Psychological and physiological factors underlying stress-induced health complaints in victims of bullying

Social defeat, genetics and microRNAs

By

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Abbreviations

3’-UTR	Three prime untranslated region
ACTH	Adrenocorticotropic hormone
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATP	Adenosine triphosphate
CCL2	Chemokine (C-C motif) ligand 2
cDNA	Complementary DNA
C/EBP	CCAAT-enhancer-binding protein
CRH	Corticotropin-releasing hormone
CX3CL1	Fractalkine
DNA	Deoxyribonucleic acid
E	Epinephrine
fMRI	Functional magnetic resonance imaging
HPA	Hypothalamic-pituitary-adrenal
HSCL	Hopkins symptom checklist
IL	Interleukin
miRNA/miR	MicroRNA
mRNA	Messenger RNA
NLRP3	NACHT, LRR and PYD domains-containing protein 3
NAQ	Negative acts questionnaire
NE	Norepinephrine
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NMDA	N-methyl-D-aspartate
NRS	Numeric rating scale
POMC	Pro-opiomelanocortin
qPCR	Quantitative polymerase chain reaction
rmAVONA	Repeated measures analysis of variance
RNA	Ribonucleic acid
SNP	Single nucleotide polymorphism
SNS	Sympathetic nervous system
TNF	Tumor necrosis factor

List of publications

The present thesis is based on the following publications:

Paper I. Nielsen, M. B., Gjerstad, J., **Jacobsen, D. P.** & Einarsen, S. V. 2017. Does Ability to Defend Moderate the Association between Exposure to Bullying and Symptoms of Anxiety? *Front Psychol,* **8,** 1953.

Paper II. **Jacobsen, D. P.**, Nielsen, M. B., Einarsen, S. & Gjerstad, J. 2018. Negative social acts and pain: evidence of a workplace bullying and 5-HTT genotype interaction. *Scand J Work Environ Health,* **44,** 283-290

Paper III. **Jacobsen, D.P.**, Eriksen, M.B., Rajalingam, D., Nymoen, I., Nielsen, M.B., Einarsen, S., Gjerstad, J. Exposure to workplace bullying, microRNAs and pain; evidence of a moderating effect of miR-30c rs928508 and miR-223 rs3848900. Submitted

Summary

Workplace bullying is a strong social stressor that may give rise to subjective health complaints, such as anxiety and pain. This thesis addresses individual susceptibility towards this form of social stress, including coping and genetic factors. In a probability sample of Norwegian employees, the effect of perceived helplessness and serotonin transporter genotype on the relationship between exposure to bullying and the development of subjective health complaints was examined. In rats, a resident-intruder paradigm was implemented to screen for stress-induced changes in circulating miRNA levels. The effect of human polymorphisms in the genes encoding the top three miRNAs from the resident-intruder paradigm was also investigated. The human data revealed that the inability to defend oneself against bullying behaviors increased anxiety. However, this effect was limited to subjects exposed to low levels of negative social acts. The serotonin transporter *SLC6A4* length polymorphism in combination with rs25531 had a moderating effect on the relationship between exposure to bullying behaviors and pain. For subjects with the L_AL_A genotype this relationship was significantly stronger than for subjects with the $SL_G/SL_A/L_AL_G$ genotypes. The animal data demonstrated stress-induced health effects, including reduced weight gain, hypothalamic-pituitary-adrenal (HPA) changes and upregulation of circulating miR-146a, miR-30c and miR-223. Follow-up of these findings in the human cohort showed that subjects with the miR-30c rs928508 GG genotype had a significantly stronger relationship between exposure to bullying behaviors and pain than other subjects had. The same was observed in men with the miR-223 rs3848900 G genotype, as compared to men with the A genotype. In summary, high exposure to bullying behaviors at the workplace seems to increase anxiety among those targeted regardless of the perceived ability to defend oneself against such behaviors. Moreover, our findings indicate that the development of pain in victims of bullying may involve serotonin signaling. Interestingly, the present study supports the hypothesis that bullying-induced pain is regulated, or mediated, by miRNAs such as miR-30c and miR-223. Taken together, the present data show that coping and genetic factors may influence vulnerability towards bullying behaviors.

1. Introduction

The definition of the term “stress” has been debated for a long period of time. It may refer to an environmental challenge to an organism’s homeostasis, or it may refer to the biological response to such a challenge (1). Throughout this thesis, “stress” refers to the former. Exposure to stress may induce both psychological and physiological changes, depending on the subject’s appraisal of the situation (2). Eustress refers to stress exposure that is perceived as manageable and which induces a feeling of hope, meaningfulness and vigor. Eustress is positively correlated with health and well-being (3). Distress is the opposite. It refers to a negative state in which coping and adaptation mechanisms fail to return the subject to homeostasis. The intensity and the duration of the stress exposure may influence the transition to such a maladaptive state. For instance, previous studies show that bullying – a strong form of prolonged and systematic stress exposure – is highly associated with distress (4) and several subjective health complaints (5).

1.1 Workplace bullying

Workplace bullying is defined as a situation in which an employee persistently is on the receiving end of negative actions from one or several others at the workplace, and where the employee has difficulties defending him- or herself (6). This helplessness reflects a perceived power imbalance in the victim-bully relationship. The transactional model of stress and coping describes the dynamic interplay between the stressor and the subject’s appraisal of the situation underlying the development of negative health outcomes (7). According to this model, people who feel they are able to defend themselves against negative social acts are less affected by such exposure than people who feel they are unable to defend themselves.

Notably, a person’s ability to cope with a given stressor may be affected by previous stress exposure. This phenomenon was first observed in dogs, where those who could not escape painful electrical shock developed an inability to cope with subsequent aversive stimuli of a different nature (8). Such learned helplessness, i.e., fatalism and

resignation following prolonged uncontrollable stressful exposure, has been thoroughly documented in humans as well (9, 10). Thus, the power imbalance that per definition exists in a victim-bully relationship may only be enhanced over time if left unchallenged – underscoring the importance of early intervention.

The reported prevalence of negative social acts at the workplace is between 5 and 25 percent (11-13). Common negative social acts include verbal harassment, spreading of rumors and social exclusion (14) – stressors with clear neural correlates. Previous fMRI studies have shown that verbal abuse (15) and interpersonal rejection (16) may induce long lasting changes in the amygdala, while social exclusion may induce increased activity in the anterior cingulate cortex (17). Through such influences on central processes, bullying may affect emotional and physical health. Even when controlling for other psychosocial stressors at work, it is clear that bullying constitutes a serious problem with regard to employee health in contemporary working life (18, 19). In particular, bullying-induced anxiety, depression (6) and chronic pain (20) have severe impact on individual well-being as well as on society at large (21). Interestingly, a growing body of evidence indicates that the association between bullying and subjective health complaints is mediated by the neuro-immune interface.

1.2 Inflammation

Inflammation is a general defense mechanism initiated by the innate immune system in response to possibly harmful stimuli to the body – usually injury, pathogens or irritants (22). Inflammation includes local vasodilation (23), release of signaling molecules (e.g. cytokines and chemokines) and movement of white blood cells out of the circulatory system and into the inflamed tissue (24). The cytokine cascade and immune cell interactions that orchestrate the inflammatory response are tremendously complex. However, simply put, inflammation is normally initiated by activated resident immune cells. For instance, a common response to extracellular ATP – indicating cell damage – is activation of the NF-κB transcription factor (25) and an upregulation of the NLRP3 inflammasome (26). The ensuing upregulation of cytokines (27), chemokines (28) and surface adhesion molecules (29, 30) drives circulating monocytes and neutrophils to the

site of injury. Infiltrating, as well as resident, immune cells then carry out the acute phase inflammatory response, which involves clearing out damaged cells and pathogens as well as initiating tissue repair.

Monocytes are a type of bone marrow-derived white blood cell that may produce cytokines and differentiate into macrophages (31, 32). Macrophages are mononuclear phagocytes that are found in nearly all tissues, where their function may range from pro- to anti-inflammatory – from host defense to resolution of inflammation and tissue repair – depending on signals from their microenvironment (33). Microglia are macrophage-related cells found in the brain and spinal cord, where they are involved in housekeeping and constitute the main line of defense against damaged cells and infectious agents within the central nervous system (34). Neutrophils are the most abundant cell type in human blood. They are derived from the same myeloid progenitor cells as monocytes, but unlike monocytes they are polymorphonuclear. Neutrophils play a crucial role in acute inflammation through cytokine release and phagocytosis (35).

Under normal, healthy circumstances, inflammation is resolved by clearing out immune cells through apoptosis and phagocytosis (36). In some cases, however, inflammation may become chronic – lasting for several months or years. Such failure to terminate the inflammatory response is maladaptive and is linked to many debilitating diseases. For instance, previous studies show that cardiovascular disease (37), chronic pain conditions (38) and mental health disorders (39) may be due to prolonged inflammation. Understanding what may cause such chronification is of great importance to public health.

1.3 Stress and inflammation

Acute stress elicits an immediate autonomic and a slower, longer lasting, neuroendocrine response – i.e., activation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis, respectively. The immediate neuronal norepinephrine stimulation of motor and hormonal tissues activates the “fight or flight” response. This includes elevated heart rate, vasodilation near muscles and increased

circulating glucose and epinephrine. This state of alertness is usually quickly terminated by the parasympathetic reflex arc.

The slower activation of the HPA axis begins with secretion of corticotropin-releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus. This hormone induces corticotropic cells in the anterior lobe of the pituitary gland to release adrenocorticotropic hormone (ACTH) into the blood stream. ACTH, in turn, binds its receptor in the adrenal gland, which leads to elevated levels of circulating glucocorticoids (40). Peak plasma glucocorticoid levels occur tens of minutes following stress exposure (41). These glucocorticoids facilitate the breakdown of glycogen and aid metabolism of fat and protein, thereby increasing available energy.

Norepinephrine and glucocorticoid signaling have been shown to have short-term anti-inflammatory effects (42, 43). However, recent findings in animals indicate that prolonged sympathetic signaling induced by chronic psychological stress may actually recruit microglia and induce neuroinflammation (44). In fact, central blockage of epinephrine and norepinephrine receptors prevented the activation of microglia and the development of anxiety-like behavior in mice following repeated social defeat (45). In addition, the sympathetic nervous system may be responsible for inducing IL-6 release from central neurons (46, 47). This cytokine regulates macrophage differentiation (48), neutrophil recruitment (49) and is capable of crossing the blood brain barrier (50). Previous findings in rats suggest that IL-6 also is part of the hormonal response to stress by showing that IL-6 release from circulating immune cells may be regulated by the HPA axis (51). Accordingly, elevated plasma IL-6 levels have been observed in humans following traumatic events (52) and experimental stress (53, 54).

In the periphery, the autonomic nervous system innervates immunological tissues like the bone marrow (55, 56), lymph nodes (57, 58) and spleen (59). In the bone marrow and spleen, sympathetic signaling may activate transcription factors like NF-κB and change the inflammatory profile of myeloid cells (60-62). For instance, norepinephrine may induce glucocorticoid resistance in monocytes by reducing expression and efficacy of type II adrenal steroid receptors, thereby making these cells unresponsive to the anti-inflammatory effects of glucocorticoids (63-65). Furthermore, norepinephrine may

induce white blood cell egress from the bone marrow (66-68), and monocyte migration into the brain (69). The surface adhesion molecules CCL2, CX3CL1 (70, 71) and IL-1 receptor type 1 (72) may be responsible for the stress-induced migration of monocytes specifically into brain regions like the prefrontal cortex, hippocampus and amygdala – associated with social behavior (73), memory (74) and emotions (75), respectively.

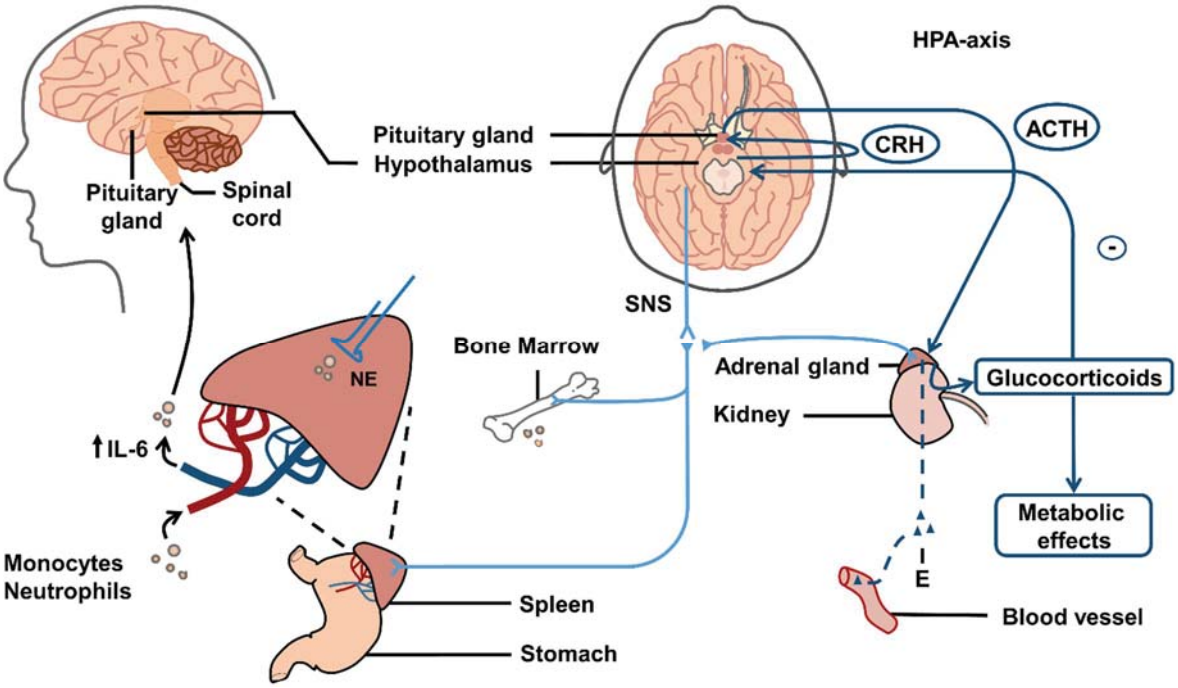

Figure 1. Schematic illustration of the neuro-immune interface and the HPA axis. Acute stress activates the sympathetic nervous system (SNS), which leads to epinephrine (E) release from the adrenal gland. Sympathetic norepinephrine (NE) signaling also stimulates immune cell egress from the bone marrow and alters the properties of monocytes in the spleen. Monocytes and circulating cytokines (e.g. IL-6) may in turn affect central processing. The hormonal response to stress is initiated by CRH released from the hypothalamus, which stimulates corticotropic cells in the pituitary gland. The subsequent release of ACTH induces glucocorticoid secretion from the adrenal gland. Glucocorticoids then negatively regulate HPA axis activity. (Illustrated by Eriksen, M.B.)

1.4 Anxiety and depression

Depression most commonly refers to a deep feeling of sadness and a lack of interest or pleasure, while anxiety refers to feelings of tension, worry and fear. The etiology of these mental health problems is complex, but seems to involve dysregulation of brain regions responsible for mood, motivation, reward and emotions. Decreased activity in

the nucleus accumbens (76, 77), lateral septum (78, 79), anterior cingulate cortex (80), hippocampus (81) and prefrontal cortex (82, 83) has been associated with anxiety and depression. In addition, increased activity in the amygdala – possibly associated with increased negative emotion – has been observed in individuals with depression (77, 84).

Previous findings suggest that these alterations in brain activity may be due to inflammation (39). For instance, post mortem brain analyses of subjects who suffered depression have revealed dysregulation of microglia (85-88), infiltration of monocytes into the anterior cingulate cortex (89) and altered expression of genes involved in inflammation (90). Moreover, microglia activation and monocyte migration into the central nervous system have been shown to be crucial for the development of anxiety-like behavior in animals (45, 69, 70).

Peripheral inflammation may also play a role in the development of mental health issues (91). Previous studies have shown that anxiety and depression may be associated with increased neutrophil-lymphocyte ratio (92-94) indicating increased activity and proliferation of innate immune cells. In addition, analysis of peripheral inflammatory markers in the blood of patients suffering from these mental health disorders has revealed an upregulation of cytokines like IL-6, IL-1β and TNF (95-98). Interestingly, in mice, knockout of IL-6 prevented the development of stress-induced anxiety (99), while knockout of IL-1 receptor type 1 prevented the development of stress-induced glucocorticoid resistance in splenic immune cells (100).

Understanding the role of inflammation in the development and maintenance of mental health disorders might help produce new pharmaceutical therapies to complement psychological treatment. Currently, the most commonly prescribed drugs to treat anxiety are benzodiazepines, which enhance inhibitory neuronal signaling and have sedative sleep-inducing properties. Long-term use of such drugs may induce increased tolerance and ultimately dependence and addiction (101, 102). This is a serious issue, as anxiety and depression affect about 20% of people in the western world (103-105), and account for about 20% of sickness absence (104, 106, 107). Moreover, anxiety and depression are highly comorbid with physical symptoms (108), such as chronic pain (109-111).

1.5 Pain

"Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" (112). Nociception, in contrast, is defined as "the neural processes of encoding and processing noxious stimuli". Pain differs from nociception as it depends on experience. Under normal conditions, pain perception is initiated when high threshold nociceptors are activated in the periphery. These primary nociceptors lead to the spinal cord where the signal is transmitted across spinal synapses by glutamate (113) and substance P (114). These neurotransmitters depolarize the postsynaptic membrane by binding to AMPA receptors, NMDA receptors, metabotropic glucocorticoid receptors and neurokinin 1 receptors. Spinal nociceptive-specific and wide dynamic range neurons then mainly relay the signal to the thalamus, from where neurons project to what is known as the pain matrix. The primary and secondary somatosensory cortices are responsible for the discriminative pain experience (115, 116), while the insular cortex, cingulate cortex (117, 118) and amygdala (119) are responsible for the affective-motivational dimension of pain. Enhanced pain perception due to central changes such as increased synaptic efficacy (120), reduced inhibition (121) and activated microglia and astrocytes (122) is known as central sensitization (112, 123). Peripheral sensitization, on the other hand, refers to increased responsiveness, and/or spontaneous activity in primary afferent nociceptors.

Such changes – either peripheral or central – are responsible for the development of pain conditions, and may be induced by a variety of mechanisms, of which inflammation seems to be especially prominent. For instance, previous studies show that IL-6, IL-1β, TNF and a variety of other pro-inflammatory cytokines may sensitize primary afferent nociceptors as well as central components of the pain matrix (124-130). Moreover, inflammation has been associated with long lasting pain conditions like lumbar radicular pain (131-133), arthritis (134), chronic widespread pain (135, 136) and fibromyalgia (137).

About 20% of people in the western world are afflicted with chronic pain conditions (138-140), accounting roughly for 30-35 % of sickness absence (141). As with anxiety, the currently prescribed drugs to alleviate pain are not a viable long-term strategy to combat this public health concern (142, 143). Once again, understanding the biological and psychological mechanisms underlying pathophysiology following prolonged exposure to social stress is of great importance. Taken together, anxiety, depression and chronic pain are responsible for about half of sickness absenteeism, are highly comorbid, and are all associated with stress and inflammation.

1.6 Genetic factors

Genetic variation between individuals refers to single nucleotide polymorphisms (SNPs), length polymorphisms, insertions, deletions and rearrangements in the genetic code. Polymorphism may occur within or outside of coding regions, thereby possibly affecting function or expression, respectively. A majority of genes are thought to be under the influence of genetic variation. In fact, roughly 85% of exons are within 5 kb of at least one of the 1.4 million SNPs present in the human genome (144, 145). Previous findings show that such individual genetic differences may affect susceptibility towards stress (146). Moreover, the heredity of mental disorders like anxiety and depression is reportedly about 50% (147-151), while about 60% of the variance in individual pain sensitivity is linked to genetics (152).

Polymorphisms influencing the expression of the serotonin transporter – which transfers serotonin back into the presynaptic neuron – have been especially well studied. Serotonin is a neurotransmitter that activates and regulates the HPA axis in the hypothalamic paraventricular nucleus (153-155). Moreover, serotonin may affect spinal nociceptive transmission directly (156, 157), and modulate the analgesic effect of exogenous opioids (158, 159). Accordingly, factors influencing function or efficacy of serotonin signaling have been linked to anxiety (160), depression (161, 162) and pain (163-165).

The *SLC6A4* length polymorphism (166) and the rs25531 A>G SNP (167) are by far the most studied polymorphisms regarding the serotonin transporter gene. Moreover, these polymorphisms are in very high linkage disequilibrium (168); the rs25531 G allele almost always coincides with the long (L) version of the 5-HTTLPR length polymorphism. Previous findings show that the short (S) version of the promotor as well as the G allele are associated with lower expression of the serotonin transporter gene, as compared to their counterparts (169). Subjects with the L_AL_A genotype are presumed to have the highest expression, whereas those with the SS genotype are presumed to have the lowest expression.

1.7 microRNA

MicroRNAs (miRNAs) are small, noncoding RNA molecules that play important gene regulatory roles in animals and plants. They are 21-22 base pairs long and specifically suppress gene expression by binding to complementary 3'-UTRs of mRNA molecules, destabilizing them and preventing translation into proteins (170-173). If the miRNA molecule is combined with proteins from the Argonaute family into an RNA-induced silencing complex, such binding may even induce degradation of the target mRNA molecule in a process called RNA interference (174, 175). With a growing number of identified miRNAs, each with multiple potential targets, it is estimated that about 60% of human genes are under miRNA regulation (176).

Extracellular miRNAs are found in most bodily fluids, where they play an important role in intercellular signaling (177, 178). Previous findings in humans have shown dysregulation of such miRNAs in blood following stress (179). Moreover, animal studies show that sympathetic (180) and glucocorticoid (181) signaling may directly affect the miRNA expression profile in target tissues. As many miRNA targets are involved in inflammation (182-184), it is not surprising that several miRNAs also have been associated with anxiety (185, 186), depression (187-189) and pain (190). Taken together, regulatory miRNAs may be the means by which the neuro-immune interface influences health and well-being in response to prolonged stress exposure.

In addition to post-transcriptional inhibition of gene expression, miRNAs may also affect epigenetic machinery and regulation. For instance, RNA interference may be involved in the methylation of specific genes (191). Previous findings in human cells show that the specific binding of short interfering RNA molecules to complementary genomic DNA may induce methylation of CpG islands in a DNA-methyltransferase dependent manner (192). Moreover, miRNAs may directly downregulate a number of DNA methylation (193) and histone acetylation (194) enzymes, thereby affecting de novo generation and maintenance of methylation patterns. Interestingly, RNA interference may also directly influence DNA structure – an important part of epigenetic regulation of gene expression (195, 196). These findings suggest that miRNAs may induce long lasting changes in expression through gene silencing.

Vice versa, the expression of miRNAs may be under epigenetic control (197, 198). Currently, an estimated 11% of miRNAs are assumed to be regulated by DNA methylation (199). As each miRNA may have multiple different targets, long lasting epigenetic regulation of miRNA expression may be important for cell differentiation (200). Similarly, patterns of miRNA expression associated with pathophysiology may also be long lasting, and may persist beyond cell division, making them potentially powerful targets for therapeutic intervention.

2. Aims

The purpose of this thesis was to provide new knowledge about psychological and biological factors underlying the relationship between exposure to bullying behaviors and the development of anxiety and pain. More specifically, we aimed to:

1. Investigate the association between exposure to bullying behaviors and anxiety, moderated by the ability to defend oneself – thereby studying the importance of a perceived power imbalance in a victim-bully relationship.

2. Investigate the association between exposure to bullying behaviors and pain, moderated by the *SLC6A4* length polymorphism in combination with rs25531 – previously shown to affect the expression of the serotonin transporter gene.

3. Screen for stress-induced changes in circulating miRNAs using a resident-intruder paradigm on rats. Identify human SNPs affecting the expression of the relevant miRNAs using literature searches. Examine the moderating effect of these SNPs on the association between exposure to bullying behaviors and pain.

3. Methods

This thesis was based on both human and animal data. The cross-sectional design in the human arm of the project only allowed us to study associations between work factors and health outcomes. Therefore, rodents were used as models to study the causal relationships between exposure to social stress and physiological changes. Findings from such animal studies may often be successfully extrapolated to humans (201), but it is important to note that in some cases findings are partially or completely lost in translation (202). Thus, although animal data may provide useful insight into the cause and effect of disease states or drug therapies, such findings must be verified in humans as well (203). Below is a brief summary of the methods used – more details may be found in the respective papers.

3.1 Human Cohort

The human analyses of this thesis were performed on a random sample of the Norwegian working population. The selection of 5000 subjects between the ages of 18 and 65 from The Norwegian Central Employee Register was performed by Statistics Norway. The subjects were sent questionaires in the mail. The Negative Acts Questionnaire (NAQ), the Hopkins Symptoms Checklist (HSCL) and a numeric rating scale (NRS) were used to measure exposure to bullying, symptoms of anxiety and pain, respectively. Each item in the NAQ had options ranging from 1 to 5 (“never” to “daily”) while each item in the HSCL had options ranging from 1 to 4 (“not at all” to “extremely”). The ability to defend oneself against negative social acts was measured with a single item question. Subjects who gave consent were sent saliva kits. In paper I, 739 subjects were included. In paper II, 987 subjects were included. In paper III, 452-996 subjects were included, depending on the analysis.

Genotyping (paper II and III)

DNA was isolated from saliva samples. SNP genotyping for rs25531 (paper II), rs2910164, rs928508 and rs3848900 (paper III) was carried out on a Quantstudio 5 machine, using

custom TaqMan genotyping assays. Length polymorphisms in SLC6A4 were determined using gel electrophoresis following PCR on a Perkin Elmer GeneAmp PCR 2400 (paper II). Subjects were divided into three groups based on presumed serotonin transporter expression (paper II): low expression (SS), medium expression ($SL_G/L_AL_G/SL_A$) and high expression (L_AL_A). Regenotyping of 25% of samples was performed, with 100% concordance.

3.2 Animal study (paper III)

All animal experiments were approved by the Norwegian Food Safety Authority and performed in conformity with the laws and regulations controlling experiments and procedures on live animals in Norway.

In order to study social stress in animals, a resident-intruder paradigm was implemented. Ten Long Evans rats (500-550 g) were used as residents, each living with a sterilized companion female (250 g). Ten male Sprague Dawley rats (400-500 g) were used as intruders, and ten as controls (Janvier Labs, Le Genest St Isle – France). Animals were acclimatized to an artificial 12h light / 12 h dark cycle for two weeks before baseline (day -7). One week after baseline (day 0), intruder rats were subjected so social stress by being introduced into a resident cage for 1 hour. This procedure was repeated for 7 days (day 0-6). On day 7, the pituitary and adrenal glands were harvested. Blood samples were collected from intruder and control animals at day -7, 0, 3 and 7.

Tissue mRNA analysis

RNA was isolated from pituitary and adrenal tissue using the AllPrep DNA/RNA/miRNA Universal Kit (Qiagen), and cDNA synthesis was carried out using the qScript cDNA Synthesis Kit (Quanta). Expression of candidate genes was determined by qPCR on a StepOnePlus machine (Applied Biosciences, USA), with β-actin as an internal reference gene.

Plasma miRNA analysis

Synthetic C. Elegans miR-39-3p was spiked into 100 µl plasma samples before RNA extraction. Total RNA was then isolated using the miRNeasy serum plasma isolation kit (Qiagen). Pooled RNA samples for residents and controls were compared using the Rat miRNome miScript miRNA PCR Array containing 653 miRNAs (cat.no. MIRN-216Z, Qiagen). Expression levels were normalized to c-mir-39-3p and SNORD68. The five miRNAs with the highest difference in fold change between residents and controls were followed up in all samples, at all four time points.

3.3 Statistics

Statistical analyses were conducted with IBM SPSS 24.0 (paper I) and Stata 15 (paper II-III). The level of significance was set to $p<0.05$. In the human studies, exposure to bullying behavior and symptoms of anxiety were calculated as the mean score of the 9 items in the NAQ inventory (paper I, II and III), and the mean score of 5 items in the HSCL inventory (paper I), respectively. To investigate the moderating effect of the ability to defend oneself (paper I) and genotypes (paper II-III), regression analyses were performed in two steps. First, covariates, ability to defend/genotypes and NAQ scores were included as predictors in the regression analyses with anxiety (paper I) or pain (paper II-III) as outcome. Second, an interaction term between ability to defend/genotype and NAQ was included.

In the animal study (paper III), differences in body weight development between resident and control rats were analyzed using a two-way rmANOVA with a Greenhouse–Geisser correction. Group differences in pituitary and adrenal gene expression were analyzed using the Mann-Whitney rank sum test. The Mann-Whitney rank sum test was also used to analyze the difference between plasma miRNA from resident and control rats. After correcting for multiple testing, using a false discovery rate approach, the top 3 miRNAs – ranked by q-value – were followed up in the human cohort.

4. Results

4.1 Paper I

A significant positive correlation between NAQ score and anxiety was observed in the first step of the regression analysis. Interestingly, inclusion of the interaction term revealed a moderating role for the ability to defend oneself, with subjects lacking the ability being significantly more vulnerable than others were at lower NAQ scores. The difference between those who felt capable of defending themselves, and those who did not, gradually decreased with increasing NAQ score, and ultimately disappeared for subjects exposed to high levels of bullying behaviors.

4.2 Paper II

A significant positive correlation between NAQ score and pain was observed in the first step of the regression analysis. Inclusion of the interaction term between serotonin transporter genotype and NAQ revealed a moderating role for the *SLC6A4* length polymorphism in combination with rs25531. Subjects with high expression (L_AL_A) were significantly more vulnerable to bullying behaviors than subjects with medium ($SL_G/L_AL_G/SL_A$) expression were. No difference was observed between subjects with high and low (SS) expression.

4.3 Paper III

The resident intruder paradigm significantly attenuated weight gain in intruder rats compared to controls. Moreover, reduced pituitary POMC (ACTH precursor) and adrenal NR3C1 (glucocorticoid receptor) expression was observed. Regarding circulating miRNAs, the top 3 upregulated miRNAs, ranked by q-value, were miR-146a, miR-30c and miR-223.

In the human cohort, the relationship between NAQ and pain was amplified for subjects with the miR-30c rs928508 GG genotype, as compared to subjects with AG or AA. The same was observed for men with the miR-223 rs3848900 G genotype, as compared to other men.

5. Discussion

The data from the human cohort showed that exposure to negative social acts was positively correlated with subjective health complaints. In victims of bullying behavior, anxiety was influenced by the ability to defend oneself (paper I), whereas pain was moderated by the serotonin transporter genotype. Upregulation of circulating miR-146a, miR-30c and miR-223 was shown after 7 days of social stress in rats (paper III). Interestingly, miR-30c and miR-223 genotype moderated the relationship between exposure to bullying behaviors at the workplace and pain (paper III).

5.1 Bullying and subjective health complaints

Previous studies have shown that exposure to workplace bullying is associated with a number of health complaints, from psychiatric disorders (4, 6) to somatic ailments (204-206). This association may be due to dysregulation of the amygdala and anterior cingulate cortex following social stress (15-17). As mentioned above, these brain regions have been associated with mental health (80, 84), and are responsible for the affective-motivational aspect of pain sensation (118, 119). Moreover, a growing body of evidence shows that inflammation may be a link between stress exposure and subjective health complaints. For instance, animal models of stress have demonstrated microglial activation (45) and immune cell migration to the brain (69), as well as increased levels of circulating cytokines (207). In humans, brain analyses of suicide victims who suffered depression have revealed activated microglia (86) and monocyte migration into the brain (89). In addition, anxiety (98), depression (96) and chronic pain (38) have all been associated with increased levels of circulating cytokines – e.g., IL-6.

In accordance with previous studies on bullying outcomes, the present work shows that exposure to bullying behaviors is correlated with anxiety (paper I), and general pain intensity (paper II-III). Here we show that the relationship between social stress and subjective health complaints may be moderated by coping mechanisms and genetic factors.

5.2 Ability to defend oneself (paper I)

The presence of a perceived power imbalance is a crucial aspect of bullying – even part of the definition. Thus, we hypothesized that in our cohort, the relationship between exposure to bullying behaviors and anxiety would be moderated by the subject's ability to defend him- or herself. This would be in accordance with the transactional model of stress and coping (7), and was also what we observed at low levels of exposure to negative social acts. However, as the exposure increased, the difference between subjects able to defend themselves and those unable to, decreased.

The observed ceiling effect of the perceived ability to defend against bullying behaviors in a victim-bully relationship could be due to incongruence – i.e., the victim's positive self-perception does not conform with the reality of being exposed to severe levels of bullying behaviors over a prolonged period of time. Such dissonance could have detrimental health effects in itself (208). Moreover, previous studies show that even when the victim appraises the situation as controllable, severe exposure to bullying behaviors still has detrimental health effects (209, 210). In accordance with these earlier reports, the current finding suggests that systematic exposure to negative social acts over an extended period of time differs fundamentally from how stress exposure is described in psychological models. In other words, individual variation in the outcomes of bullying must be explained by other factors than psychological coping mechanisms. In the present thesis, biological differences have been investigated as one possible explanation.

5.3 Serotonin genotype (paper II)

An extensive body of evidence shows that serotonin transporter gene polymorphisms may affect stress, anxiety, depression and pain mechanisms. Several lines of evidence indicate that low expression of the serotonin transporter is associated with reduced stress resiliency (211). Others report either no interaction between serotonin transporter genotype and vulnerability towards stress (212) or even the opposite – high

expression of the serotonin transporter being associated with trauma-induced depression (213). Reports of gender differences further complicates matters (214). The same is true for pain susceptibility. Both low (215) and high (163) expression of the serotonin transporter have been associated with heightened pain intensity. Likewise, both S-carriers (216) and L_AL_A subjects (217) are prone to exhibit increased emotional pain facilitation.

In the present thesis, we show that subjects with the L_AL_A genotype were more vulnerable to bullying behaviors, with pain as outcome, than subjects with the $SL_G/L_AL_G/SL_A$ genotype. Interestingly, subjects with the SS genotype were more similar to subject with L_AL_A than to subjects with $SL_G/L_AL_G/SL_A$. Thus, it is tempting to speculate that there might be a trade-off, where both very low and very high expression of the serotonin transporter may be detrimental. More research needs to be done in order to establish such a relationship, but this could explain the high number of conflicting data in the literature.

5.4 Resident intruder paradigm (paper III)

Previous studies using animal models have shown altered behavior (40), attenuated bodyweight gain (218), dysregulation of key components of the HPA axis (219) and altered immune profile (61, 220, 221) following prolonged exposure to stress. In accordance with these findings, our resident intruder paradigm induced lower weight gain as well as reduced pituitary POMC and adrenal NR3C1 expression, indicating that the implemented paradigm successfully induced physiological changes.

The miRNA analysis revealed a persistent stress-induced upregulation of three circulating anti-inflammatory miRNAs: miR-146a, miR-30c and miR-223. Previous findings indicate that miR-146a may be upregulated by the inflammatory gene regulatory protein complex NF-κB (182). Therefore, the observed upregulation of miR-146a may indicate an activation of NF-κB following social stress. However, miR-146a is not part of the canonical pro-inflammatory NF-κB response, but rather part of a negative feedback mechanism. For instance, the 3’ UTR of IL-1 receptor-associated kinase 1, as

well as that of TNF receptor-associated factor 6 are predicted targets of miR-146a (182). Moreover, it has been suggested that miR-146a may serve as a molecular regulator in macrophage polarization by targeting Notch1 (222) – a transmembrane signaling protein involved in IL-6 and TNF regulation (223).

In humans, circulating miR-30c has been shown to be mainly located within microparticles derived from macrophages (224). Within macrophages, miR-30c negatively regulates a pro-atherosclerosis pathway by targeting caspase 3 and reducing IL-1β release. This suggests that the observed upregulation of miR-30c may, similar to miR-146a, negatively regulate stress-induced inflammation. Moreover, DNA methyltransferase 3B has been identified as a possible target for miR-30c (225). Thus, upregulation miR-30c may dampen generation of de novo methylation patterns in stressed animals.

MiR-223 is located on the x-chromosome, where it is regulated by two C/EBP binding sites in its promotor region (226, 227). This hematopoietic specific miRNA has a crucial function in regulating granulocytic differentiation by targeting the transcription factor Mef2c – which promotes myeloid progenitor proliferation (228). Similar to miR-146a, miR-223 also suppresses the pro-inflammatory NF-κB pathway (229). Other anti-inflammatory functions of miR-223 are negatively controlling NLRP3 inflammasome activity (230) as well as reducing pro-inflammatory cytokine production and macrophage polarization by selectively downregulating Pknox1 (231). In accordance with our current findings, upregulation of miR-223 has previously been observed in serum and amygdala samples from animals following repeated immobilization stress along with tail shock (232). Taken together, the observed upregulation of miR-146a, miR-30c and miR-223 in plasma following repeated exposure to social stress may constitute an important negative feedback to stress-induced inflammation.

5.5 miRNAs in human cohort (paper III)

In accordance with the proposed protective role of miRNA-146, downregulation of this miRNA has previously been observed in the prefrontal cortex of suicide victims who

suffered depression (189). Thus, the miR-146a rs2910164 SNP, located on the passenger strand of pre-miR-146a, could in theory affect bullying resiliency. In fact, the rare C allele has been shown to halter nuclear processing of the transcript, effectively reducing the expression of mature miR-146a by half (233). However, the current work showed no moderating effect of miR-146a rs2910164 genotype on the relationship between prolonged exposure to bullying behaviors and pain.

The miR-30c rs928508 SNP, located on the flanking region of miR-30c, may also affect gene expression. Previous studies show that the rare G allele inhibits the transition from pri-miR-30c to pre-miR-30c, thereby reducing the expression of mature miR-30c (234, 235). In the present thesis, we demonstrated a moderating effect of miR-30c rs928508 genotype on the relationship between exposure to bullying behaviors and pain. Interestingly, subjects with the GG genotype – associated with the lowest expression – were significantly more vulnerable to bullying behaviors, with pain as outcome, as compared to subjects with AG or AA genotype. This finding is in accordance with the suggested protective role of miR-30c.

One of the C/EBP binding sites in the promotor region of miR-223 contains the miR-223 rs3848900 SNP (227). The effect of this SNP has not previously been studied, but because of its location it is tempting to speculate that it affects miR-223 expression. Interestingly, in men, the rare G allele of this SNP appeared to enhance the association between bullying and pain. Therefore, it is possible that potentially altered miR-223 expression brought forth by miR-223 rs3848900 could affect the development of stress-induced inflammation. Moreover, as miR-223 is known to target central AMPA receptors and NMDA receptors, it is possible that altered miR-223 expression could have a direct effect on pain signaling (236). In fact, application of miR-223 onto the dorsal nerve roots of rats has previously been shown to attenuate neuronal signaling in spinal pain pathways (237).

The frequency of the miR-146a rs2910164 C allele was very low. A larger cohort may be needed in order to show the effect of this SNP. However, both the miR-30c and miR-223 SNPs moderated the association between stress – i.e., exposure to bullying behaviors – and pain. These two miRNAs could be future targets of pharmacological interventions.

Moreover, miR-30c and miR-223 could be used as biomarkers of the physiological impact of traumatic experiences. However, more research needs to be done in order to establish the relationship between exposure to environmental stressors and these miRNAs, as well as to determine their mechanistic role in the development of pathophysiology.

5.6 Further studies

The human arm of the project was based on a probability sample of the Norwegian working population. In order to validate the current findings, replication in a different cohort is needed. It would be interesting to accomplish this either by collaborating with other groups or by generating a new cohort. Previous studies have shown that the relationship between workplace bullying and mental health is bidirectional (5). Thus, longitudinal studies with causal interpretation should be performed in order to establish the roles of being able to defend oneself, the serotonin transporter genotype, miR-30c rs928508 and miR-223 rs3848900.

As in earlier studies, subjects were divided into 3 groups based on serotonin transporter genotype: SS, $SL_G/SL_A/L_AL_G$ and L_AL_A (163). Ideally, this analysis should be performed with all 5 genotypes separately. This would require a larger cohort than the one generated in the present work, but would perhaps give additional insights into how serotonin uptake efficacy influences the relationship between bullying behaviors and pain.

In the present thesis, a causal relationship between exposure to social stress and miR-146a, miR-30c and miR-223 expression in the plasma of rats was shown. Our group has just finished another series of behavioral experiments – this time isolating monocytes from the blood, bone marrow and spleen after one week of social stress. In these cells, we aim to replicate the present data. Moreover, we aim to identify intracellular miRNAs involved in the stress response.

We also plan to perform experiments on THP-1 cells in order to investigate mechanistic properties. For example, we would like to stimulate these cells with norepinephrine or stress-induced cytokines, like IL-6, and investigate the expression of our miRNAs of

interest. This would directly link their upregulation to the activation of the sympathetic nervous system or to the ensuing inflammatory response. In addition, it would be interesting to investigate the moderating effect of our miRNAs of interest on gene expression by transfecting them into THP-1 cells, and then stimulate these cells with either norepinephrine or cytokines.

In the present work, we assume an effect of the miR-223 rs3848900 SNP because of its location in the transcription factor C/EBP binding site of the miR-223 promotor region. The actual function of this SNP has not yet been investigated. This could be achieved using a similar approach as was previously used to investigate the functional effects of miR-146a rs2910164 and miR-30c rs928508 (233, 234): constructing two different plasmids – one for each allele – and transfecting these into cells that do not normally express miR-223. Then, gene expression analysis following C/EBP stimulation would reveal possible functional effects on transcription. Moreover, using human samples (e.g. blood, tissue) it would be interesting to study in vivo effects of miR-223 rs3848900 on miR-223 expression.

For ethical reasons, a human model could not be used to investigate the causal relationship between prolonged exposure to negative social acts and miRNA expression. Hence, an animal model was used. However, there are in fact established paradigms that could be used to study the effect of stress in humans directly. One example is the "Trier social stress test", based on anticipation and performance in front of an audience in a controlled setting. The Trier social stress test has been shown to induce a significant increase in heart rate, as well as an upregulation of ACTH and cortisol (238). Interestingly, upregulation of cytokines, like IL-6, has also been observed in subjects who underwent the Trier social stress test (239, 240). Using this test would make it possible to investigate causal miRNA changes in human plasma following psychosocial stress.

6. Conclusions

1. In a probability sample of the Norwegian working population, a significant positive correlation between exposure to bullying behaviors at the workplace and anxiety was observed. The protective effect of the perceived ability to defend oneself against such behaviors was limited to subjects exposed to low levels of negative social acts. These results suggest that psychological features such as the ability to defend oneself have a ceiling effect. When a victim is exposed to high levels of negative social acts at the workplace, perceived individual coping has limited to no impact on mental health outcomes.

2. In the same cohort, a significant positive correlation between exposure to negative social acts and pain was observed. This relationship was moderated by the serotonin transporter *SLC6A4* length polymorphism in combination with rs25531. Subjects with the L_AL_A genotype had a significantly stronger relationship between exposure to bullying behaviors and pain than subjects with the $SL_G/SL_A/L_AL_G$ genotype. These results suggest that the development of pain following social stress, such as bullying, may be dependent on physiological processes linked to serotonin signaling, and that high expression of the serotonin transporter enhances the development of stress-induced pain.

3. The animal data showed that repeated social defeat caused clear physiological effects: attenuated weight gain and reduced pituitary POMC and adrenal Nr3c1 expression. Regarding the miRNA screening, one week of social stress exposure induced an upregulation of miR-146a, miR-30c and miR-223 in plasma. The human data demonstrated that the relationship between exposure to bullying behaviors and pain was moderated by miR-30c and miR-223 genotype. Subjects with the miR-30c rs928508 GG genotype had a significantly stronger relationship between exposure to bullying behaviors and pain. The same was observed in men with the miR-223 rs3848900 G genotype, as compared to men with the A genotype. Taken together, these results support the hypothesis that stress-induced pain may be regulated, or mediated, by miRNAs such as miR-30c and miR-223.

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Does Ability to Defend Moderate the Association between Exposure to Bullying and Symptoms of Anxiety?

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In the context of workplace bullying, the ability to defend refers to whether or not a target feels able to deal with those negative behaviors that typically constitute bullying. The aim of this study was to determine whether the perceived ability to defend oneself moderates the association between exposure to bullying behaviors at work and symptoms of anxiety as predicted by the definition of workplace bullying. It was hypothesized that exposure to bullying behaviors would be more strongly related to symptoms of anxiety among targets feeling unable to defend oneself than among targets who do feel that they are able to defend themselves in the actual situation. This survey study was based on a probability sample of 1,608 Norwegian employees (response rate 32%). Only respondents exposed to at least one bullying behavior were included (N = 739). In contrast to hypothesis, the findings showed that ability to defend only had a protective effect on the relationship between exposure to bullying behaviors and anxiety in cases of low exposure. In cases of high exposure, there was a stronger increase in anxiety among employees able to defend themselves than among those who generally felt unable to defend. Hence, the ability to defend against exposure to bullying behaviors does not seem to protect high-exposed targets against symptoms of anxiety. Organization should therefore intervene against bullying in early stages rather than relying on the individual resilience of those exposed.

Keywords: aggression, harassment, distress, power (im-)balance, health, personality

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INTRODUCTION

An extensive body of longitudinal evidence has established exposure to bullying in the workplace as a major predictor of impaired health and well-being among employees (for reviews and meta-analyses, see Nielsen and Einarsen, 2012; Nielsen et al., 2014, 2016b; Verkuil et al., 2015). Despite this interest in the individual consequences of bullying, surprisingly little is known about the variables that moderate the relationship between exposure to bullying and outcomes. Understanding moderators is highly important as it is unlikely that all targets of bullying will respond to the exposure in the same manner and to the same degree. It is far more likely that the effects of bullying are dependent upon a range of personal, situational and organizational characteristics such as personality and individual dispositions, resilience, coping strategies, social support, organizational climate, and leadership practices (Einarsen et al., 2016; Nielsen et al., 2016a).

Workplace bullying refers to the long-lasting and systematic mistreatment of an employee by other organization members. Hence, it describes a situation where an employee persistently and over a period of time, perceives to be on the receiving end of negative actions from superiors or co-workers and where the employee finds it difficult to defend him-/herself against these actions due to a real or perceived power imbalance between target and perpetrator (Olweus, 1993; Einarsen and Skogstad, 1996). Following this definition, workplace bullying takes the form a two-step process. The first step includes exposure to systematic aggression and mistreatment over time, whereas the second step comprises a power imbalance reflected through a perception of being unable to defend oneself in the actual situation (Einarsen et al., 2011; Nielsen and Knardahl, 2015). In the reminder of this article we will refer to the first step of the process as "exposure to bullying behaviors" and the second step as "ability to defend."

Considering that powerlessness is such a central aspect of the workplace bullying phenomenon (Saunders et al., 2007; Baillien et al., 2009), it is reasonable to expect that any health and well-being outcome of being exposed to bullying behaviors are conditioned by whether the target is able to defend him-/herself against the said exposure. To elucidate the role of powerlessness in the workplace bullying process, the overarching objective of the current study was to determine whether the perceived ability to defend oneself moderates the association between exposure to bullying behaviors and symptoms of anxiety, the latter being one of the most documented outcomes of bullying (Nielsen and Einarsen, 2012; Verkuil et al., 2015). In line with well-established theories on stress (i.e., the Transactional model of stress and coping and the theory of learned helplessness) we argue that exposure to bullying is more strongly associated with anxiety among targets who feel unable to defend against the bullying behaviors than among those who feel able to defend oneself. Hence, this study extends previous literature on workplace bullying and mental health by adding to the understanding of *when* and *under what conditions* exposure to incivility and other kinds of bullying behaviors relate to the health and well-being in exposed targets.

Ability to Defend as a Moderator – Theoretical and Empirical Evidence

In most definitions of workplace bullying, the target's experience of powerlessness refers to a imbalance between target and perpetrator where the target is systematically exposed to mistreatment and harassment to the point where he or she has little resources to retaliate in kind (Einarsen, 1999; Samnani and Singh, 2016). Hence, targets may in varying degrees feel able to defend themselves against the unwanted behavior of the perpetrator. This power imbalance between the two parties, shaping this inability to defend oneself, can be both formal and informal in nature (Einarsen et al., 2011). Formal imbalance may occur in cases when the target is exposed to bullying behaviors from a person in a superior position in the organizational hierarchy and may therefore exist *a priori* to the bullying situation. Informal imbalance refers to cases where the source of power are mostly based on knowledge and experience, as well as access to support from influential persons (Hoel and Cooper, 2000). Informal power imbalance may also be reflected in the target's dependence on the perpetrator(s), be it of a social, physical, economic, or psychological nature (Einarsen et al., 2011). Such powerlessness may also develop as a function of the bullying process itself as well as being a predisposition in the target (Zapf and Einarsen, 2005).

As workplace bullying by definition involves a victim-perpetrator relationship combined with a real or perceived power-imbalance between the two (Samnani and Singh, 2012), one may argue that the perceived inability to defend is a prerequisite for defining a situation as bullying. Without it the person toward whom the bullying behaviors are directed could withstand the attacks and retaliate, thus preventing the situation from further escalation (Salin, 2003a). This suggests that the ability to defend reflects some sort of individual capacity that determines whether a target can deal with the exposure to bullying behaviors and thereby also the appraisal and subsequent consequences of this exposure. Consequently, whether the target is able to defend him/herself should be a potential moderator that governs the outcomes of repeated exposure to acts of incivility and mistreatment in the workplace. To this date, this proposition has never been tested empirically.

Theoretically, the ability to defend as a potential moderator of the relation between exposure to bullying behaviors and health outcomes can be explained by well-established stress process models. In their Transactional model of stress and coping, Lazarus and Folkman (1984) proposed that the nature and severity of reactions following exposure to a given stressor are functions of a dynamic interplay between event characteristics and individual appraisal and coping processes. When a person is faced with a stressor, the person evaluates the potential threat (primary appraisal) and a judgment is made as to whether the event is positive or negative (Lazarus, 1993). As a secondary appraisal, the person evaluates how controllable the stressor is and determines whether ones available coping resources are adequate for handling and mastering the situation (Lazarus and Folkman, 1984). Consequently, following this model, the nature and severity of any outcome of bullying should be dependent upon how the target perceive the exposure to bullying behaviors and whether or not the target is able to deal with these negative acts. That is, one can expect that a target with the ability to defend intact is less influenced by the exposure compared to a target feeling unable to defend him-/herself against the said and unwanted behaviors.

Ability to defend as a moderator can also be explained by the theory of learned helplessness (Abramson et al., 1978; Abramson et al., 1980). Learned helplessness is a state of mind that may evolve when exposed to repeated and enduring painful or otherwise aversive stimuli which the targeted person is unable to escape or avoid (Maier and Seligman, 2016). This experience of being in a position in which there is no possible way to escape from harm or pain and in which an overall fatalism and resignation makes one believe that there is no point in trying to improve the situation (Nielsen et al., 2008). Extensive evidence has shown that learned helplessness is closely related to a range of health problems, including anxiety and depression (Abramson

et al., 1989; Overmier, 2002). Following these principles, a target of bullying who perceives him-/herself to be unable to defend him-/herself against the bullying behaviors of a given perpetrator should be more likely to resign into a situation of helplessness which then may lead to increased mental distress, in our case anxiousness.

While there are no previous studies that have explicitly examined the ability to defend as a potential moderator, some findings exist on individual dispositions that may reflect the ability to defend. As noted above, the Transactional Model of Stress and coping highlights a secondary appraisal where the focal person evaluates how controllable the said stressor is thereafter determines whether ones available coping resources are adequate for handling and mastering the situation (Lazarus and Folkman, 1984). Hence, once perceived coping resources may be especially relevant with regard to the perceived ability to defend. In a review of the literature on the use of coping, the coping method that appeared to consistently produce a significant improvement in a victim's conditions was finding a way to avoid the perpetrator(s) or to leave the situation (Aquino and Thau, 2009). Similarly, in a study of 224 Danish workers, Mikkelsen and Einarsen (2002b) found that generalized self-efficacy moderated the relationship between exposure to bullying and psychological health complaints, thus indicating that employees who have a strong belief in their own general abilities to handle problems, have a lower risk of reporting health complaints. However, other studies have provided non-significant (Nielsen et al., 2013b) or contradicting findings (Nielsen et al., 2008; Vie et al., 2011; Hewett et al., 2016), something that highlights the need for further research on such individual factors as potential moderators.

Aims of the Study and Hypothesis

It has previously been established that exposure to bullying in the workplace is associated with increased psychological distress (Nielsen and Einarsen, 2012; Nielsen et al., 2014; Verkuil et al., 2015). There is, however, a shortage of evidence on factors that determines when bullying behaviors is associated with distress. Perceived ability to defend is a central aspect in the very definition of workplace bullying and personal capacities are highlighted as buffering factors in both the Transactional model of stress and coping and the theory of learned helplessness. Consequently, there are strong theoretical reasons for expecting a protective effect with regard to the health outcomes of exposure to bullying. To add to the understanding of the role of the ability to defend in the bullying process the overarching aim of this study was to investigate whether the ability to defend moderates the relationship between exposure to bullying behaviors and symptoms of anxiety. Based on the abovementioned theoretical models, we expect that ability to defend has a protective effect on anxiety, leading us to put forward the following hypothesis to be tested:

H1: Perceived ability to defend oneself against exposure to bullying behaviors moderates the association between exposure and symptoms of anxiety, so that the relationship is stronger for targets who are unable to defend themselves as compared to targets who are able to defend themselves against these behaviors.

MATERIALS AND METHODS

Design and Sample

This study is based on a survey of the Norwegian working force where a random sample of 5000 employees was drawn from The Norwegian Central Employee Register by Statistics Norway (SSB). The Norwegian Central Employee Register is the official register of all Norwegian employees, as reported by employers. Criteria for sampling were adults between 18 and 60 years of age employed in a Norwegian enterprise. Questionnaires were distributed through the Norwegian Postal Service during the spring 2015, with a response rate of 32 percent. Altogether 1,608 questionnaires were satisfactory completed and included in this study. The survey was approved by the Regional Committee for Medical Research Ethics for Eastern Norway. Responses were treated anonymously, and informed consent was given by the respondents. The procedure for this study has previously been described elsewhere (Nielsen et al., 2017).

As the overarching aim of this study was to examine the interaction between exposure to bullying behaviors and ability to defend, the sample was limited to respondents who reported exposure to at least one bullying behaviors in the employed Negative Acts Questionnaire Revised ($N = 739$). Mean age in this final sample was 43.98 ($SD = 10.28$) years with a range from 21 to 61. The gender distribution was 51.4% women and 48.6% men. In total, 47.3% were married, 28.7% were common-law partner, 15.3% were unmarried, and 8.6% were widowed, separated, or divorced. Altogether 7.7% had less than 11 years of education, 33.4% had between 11 and 13 years, 31.6% had between 14 and 17 years, while 27.3% had 18 years or more. A total of 88.1% were in a full-time employment, 6% in part time employment and 5.3% were on a sick leave or occupational rehabilitation, whereas 0.6% was disabled pensioners or retired. Altogether 36.4% had a leadership position with personnel responsibilities.

Instruments

Exposure to bullying behaviors in the workplace was measured with the 9-item version of the *Negative Acts Questionnaire – Revised* (NAQ-R) inventory (Einarsen et al., 2009). NAQ-R describes negative and unwanted behaviors that may be perceived as bullying if occurring on a regular basis. All items are formulated in behavioral terms and hence focus on the mere exposure to inappropriate behaviors while at work with no references to the term bullying (Einarsen and Nielsen, 2015). The NAQ-R contains items referring to both direct (e.g., openly attacking the victim) and indirect (e.g., social isolation, slander) behaviors (Einarsen et al., 2009). The items do also distinguish between personal and work related forms of bullying (Einarsen et al., 2009). The respondents were asked to indicate how often they had been exposed to each specific item in questionnaire at their present worksite during the last 6 months. Response categories range from 1 to 5 ('never,' 'now and then,' 'monthly,'

'weekly' and 'daily'). This nine item version of the NAQ-R had a Cronbach's alpha of 0.81 in this study.

Ability to defend was measured with a single item question developed specifically for this study. The item follows the self-labeling method for assessing workplace bullying and is based on the part of the definition of workplace bullying that describes the power imbalance between the target and perpetrator (Nielsen et al., 2011). Directly following the NAQ-R, the respondents were asked "If you have been exposed to one or more of the behaviors in the list above, did you find it difficult to defend yourself against this exposure? Response alternatives were "Not exposed to any of the acts," "No, never," "Yes, once in a while," "Yes, often."

Self-labeled victimization from workplace bullying was measured with the well-established self-labeling method (Olweus, 1991; Einarsen and Skogstad, 1996; Solberg and Olweus, 2003; Nielsen et al., 2011). After being presented with the following definition: "*Bullying (harassment, badgering, niggling, freezing out, offending someone) is a problem in some workplaces and for some workers. To label something bullying it has to occur repeatedly over a period of time, and the person confronted has to have difficulties defending himself/herself. It is not bullying if two parties of approximately equal "strength" are in conflict or the incident is an isolated event*" (Einarsen and Skogstad, 1996, p. 191), respondents were asked "*Have you been subjected to bullying at the workplace during the last 6 months?*" The response categories were "no," "rarely," "now and then," "once a week," and "several times a week."

Symptoms of anxiety during the last week were measured by five items measuring typical symptoms of anxiety from the anxiety subscale in the Hopkins Symptom Checklist (HSCL-25). The HSCL is a valid and reliable (Rickels et al., 1976) self-administered instrument measuring mental distress (anxiety, depression, and psychosomatic complaints) in population surveys (Derogatis et al., 1974). Comparisons have found that shorter versions perform as well as the more extensive versions of the inventory (Strand et al., 2003). Responses were given on a four-point scale, ranging from "1 = not at all" to "4 = extremely." Example items are "Heart pounding or racing" and "Feeling fearful." Cronbach's alpha for this scale was 0.73 in the current study.

Control Variables

The following control variables were included in the study: Age, seniority at current workplace, gender, leadership responsibility, and full-time vs. other forms of employment. Although existing evidence is inconclusive, studies have established age differences in workplace bullying (De Cuyper et al., 2009). As for gender, findings show gender differences in prevalence of bullying, (Björkqvist et al., 1994; Salin, 2003b), outcomes of bullying (Rodriguez-Munoz et al., 2010; Einarsen and Nielsen, 2015; Attell et al., 2017), and ways of coping with bullying (Ólafsson and Jóhannsdóttir, 2004). Seniority at current workplace was included as a control variable since workplace bullying by definition is a long-lasting form of exposure. Respondents with relatively short seniority may therefore be less likely to perceive potential exposure as bullying. Power imbalance is another defining aspect of bullying. To account for formal power imbalance, we adjusted for whether or not the respondents had a leadership position at the workplace. Finally, as persons with a full-time position spend more time at the workplace, and therefore should have a higher risk of negative social interactions, compared to employees with part-time employment, we adjusted for employment status.

Statistical Analyses

Statistical analyses were conducted with IBM SPSS 24.0. The level of significance was set to $p < 0.05$. For all measurement inventories, summary scales were calculated on the basis of a mean-score of their respective items. Missing data in scale variables were replaced with the Hot Deck imputation procedure (Myers, 2011). This method handles missing data by substituting each missing value with an observed response from a respondent with similar characteristic. Age, gender, and leadership position were used as predefined anchor variables in the imputation procedure.

To explore main and moderating effects, we conducted a hierarchical regression analysis, to test for linear associations between exposure to bullying behaviors and symptoms of anxiety, as well as the interactive effects of exposure to bullying and the ability to defend, with regard to anxiety. The guidelines by Baron and Kenny (1986) were followed, and, in line with Aiken and West (1991), the continuous predictor variables were centered prior to the two-way interaction analysis. The SPSS macro "Interaction and simple slopes test with one continuous and one dichotomous variable" by Jason T. Newsom[1] was used to generate the regression estimates, plots, and simple slopes analyses.

RESULTS

Validity of the "Ability to Defend" Measure

The indicator of whether the respondents were able to defend themselves against exposure to bullying behavior is a newly developed measure that has not been included in any previous studies. To provide indications of its validity, the measure was therefore compared with other measures of workplace bullying. A Spearman correlation analyses showed a significant correlation of 0.34 ($p < 0.001$) between self-labeled victimization from workplace bullying and ability to defend thus indicating an overlap between the indicators and in line with major definitions of workplace bullying. As the measure of ability to defend is limited to only one aspect of workplace bullying, whereas the questions about victimization should tap all four definitional aspects (i.e., negative acts, repetition, duration, and power imbalance), a correlation of 0.34 seems reasonable. A Spearman correlation of 0.50 ($p < 0.001$) was established between ability to defend and exposure to bullying behaviors as measured by the NAQ. A follow-up one-way ANOVA showed that respondents in the "Yes, once in a while" ($M = 1.48$; $SD = 0.35$) and "Yes, often" ($M = 2.14$; $SD = 0.79$) categories reported significantly ($F = 167.50$; df = 2/734; $p < 0.001$) higher exposure to bullying

[1] http://web.pdx.edu/~newsomj/

behaviors compared to the "No, never" ($M = 1.25$; $SD = 0.22$) category of the ability to defend indicator. As it should be harder to defend against bullying with increasing exposure, this finding is in line with reasonable expectations.

Due to few cases in the "Yes, often" category ($n = 46$), there was insufficient statistical power to examine moderating effects with all three response categories of the ability to defend measure. Hence, positive responses (i.e., "Yes, once in a while" and "Yes, often") were recoded into a single category in the main correlation and regression analyses. This is in line with previous studies that have used dichotomized single item measures to assess aspects of workplace bullying (Hauge et al., 2007; Nielsen et al., 2011).

Descriptive Findings

Means, standard deviations, and intercorrelations for all study variables are displayed in **Table 1**. Altogether 42% of the respondents who reported exposure to at least one bullying behaviors felt unable to defend themselves against the exposure. Mean scores and standard deviations for exposure to bullying behaviors and anxiety were rather small, thus indicating relatively low exposure and variance in the sample. Exposure to bullying behaviors were positively correlated with symptoms of anxiety ($r = 0.29$; $p < 0.001$) and the ability to defend ($r = 0.40$; $p < 0.001$). Ability to defend was positively associated with anxiety ($r = 0.21$; $p < 0.001$).

Main and Interaction Effects

Findings from the multiple regression analyses of linear associations and interaction effects are presented in **Table 2**. For the linear association, the control variables age, gender, seniority, employment status, and leadership position explained four percent of the variance in anxiety ($R^2 = 0.04$; $p < 0.001$; $F = 5.23$; $df = 5/730$; $p < 0.001$). Being in a full-time employment ($\beta = 0.15$; $p < 0.001$) was the only significant control variable. The explained variance in anxiety increased to 12% ($R^2 = 0.12$; $p < 0.001$) when exposure to bullying behaviors ($\beta = 0.24$; $p < 0.001$) and the ability to defend ($\beta = 0.10$; $p < 0.05$) was included in the model ($F = 14.30$; $df = 7/728$; $p < 0.001$). The amount of explained variance increased significantly by one percent when the interaction term was added to the regression ($R^2 = 0.13$; $p < 0.001$; $\Delta R^2 = 0.01$; $p < 0.05$). The interaction term made a significant contribution to the explained variance ($\beta = -0.16$; $p < 0.05$), and the interaction model was significant ($F = 13.09$; $df = 8/727$; $p < 0.001$). This means that there is an interaction effect between exposure to bullying behaviors and the ability to defend against these acts with regard to symptoms of anxiety.

To examine the nature of this interaction, scores were plotted at the mean, low (1 *SD* below the mean) and high (1 *SD* above the mean) values on the indicator of bullying behaviors and for each of the two categories of the ability to defend measure. As shown in **Figure 1**, the results indicate a stronger relationship between exposure to bullying behaviors and symptoms of anxiety for targets *with* the ability to defend themselves against the bullying when compared to those who perceived themselves to be unable to defend against these acts. Follow-up analyses of simple slopes confirm this interpretation by revealing that exposure to bullying behaviors were more strongly related to symptoms of anxiety among targets who reported to be able to defend themselves ($\beta = 0.39$; $p < 0.001$) than among targets being unable to defend ($\beta = 0.20$; $p < 0.001$). The Ratio of Residual Variances in the two groups was 0.77. A ratio value between 0.67 and 1.5 does not violate homogeneity assumptions (DeSchon and Alexander, 1996). In direct contrast to our study hypothesis about a protective effect of being able to defend oneself against bullying, the results indicate a "reverse buffer association" where ability to defend oneself seems to only have a protective effect on the relationship between bullying behaviors and anxiety in cases of low exposure to bullying behaviors. When exposure to bullying behaviors is high, there was a stronger increase in levels of anxiety among respondents with ability to defend as compared to respondents unable to defend.

The findings remained consistent when the regression analyses were repeated without including control variables.

DISCUSSION

The aim of this study was to determine the impact of being able to defend oneself on the already well-established association between exposure to bullying behaviors and symptoms of anxiety. It was expected that targets with an intact ability to defend themselves against exposure to bullying behaviors would have lower levels of anxiety compared to targets that were unable to defend. In direct contrast to the hypothesis, the findings showed that ability to defend only had a protective effect against anxiety in cases of low exposure to bullying behaviors. In cases of high exposure, targets with ability to defend reported equally high levels of anxiety as targets without this ability. This finding is not only in contrast to the study hypothesis, but also to the theoretical models which constituted the background for our expectations of the ability to defend as a moderator, that is the Transactional theory of stress and coping and the theory on learned helplessness.

Although this finding goes against theoretical assumptions about individual capacities as protective resources, it is in line with some previous studies on workplace aggression showing that the moderating effect of individual factors is dependent upon the intensity of the exposure (Nielsen et al., 2008; Ilies et al., 2011; Britton et al., 2012; Reknes et al., 2016). For instance, both Vie et al. (2011) and Hewett et al. (2016) found that self-labeling as a victim of bullying (using a self-labeling question based on a definition of bullying where the ability to defend is a central aspect), influenced the impact of exposure to bullying on the targets' health in cases of low exposure to bullying behaviors only. When facing intense bullying behaviors, exposure to bullying was as strongly associated with health complaints among those who did and did not self-label as a victim of bullying. Similarly, in a longitudinal study of 1582 Norwegian nurses which examined coping styles as moderators of the association between exposure to bullying and subsequent anxiety, it was found that active goal-oriented coping was only beneficial when exposure to bullying was low (Reknes

et al., 2016). The effect diminished as the bullying intensified, something that suggests that high exposure to bullying behaviors has negative consequences for targeted employees regardless of their coping style. Systematic and ongoing exposure to bullying behaviors is something most people will have difficulties defending against and cope with irrespective of any coping resources they may poses (see Zapf and Einarsen, 2005 for a discussion).

The behavioral concordance hypothesis suggest that individuals experience negative affect when they engage in behaviors that are contrary to their nature (Ilies et al., 2011). An experience of situational incongruence may therefore be possible explanations for the finding of a reverse buffering effect of ability to defend on the relationship between bullying behaviors and anxiety. Building on a person-environment fit perspective, the situational-congruence model proposes that a person will experience more positive and less negative affect when there is congruence between a given situation and personality (Pervin, 1993). In contrast, individuals will experience heightened negative affect in situations that are incompatible with their personality characteristics (Diener et al., 1984; Ilies et al., 2011). With regard to workplace bullying and the ability to defend, it is therefore likely that anxiety will emerge as a response when the individual experience an incongruence between self-concept ("I am able to defend myself") and external exposures (exposure to bullying behaviors) as this creates an imbalance between the targets own perception of him-/herself and actual life experiences.

TABLE 1 | Frequencies, means, standard deviations (SD) and intercorrelations for study variables ($N = 737$).

	Variable	%	*M*	*SD*	1	2	3	4	5	6	7	8
1	Gender (women)	51%	–	–	–							
2	Leadership responsibility	36%	–	–	−0.23***	–						
3	Full-time employment	88%	–	–	0.15***	−0.12***	–					
4	Age	–	43.98	10.28	−0.05	0.11	−0.02	–				
5	Seniority at current workplace	–	10.84	9.12	−00.10**	0.10**	−0.03	0.46***	–			
6	Exposure to bullying behaviors	–	1.39	0.40	−0.03	−0.02	0.03	−0.01	−0.06	**0.81**		
7	Anxiety	–	1.43	0.42	−0.06	−0.05	0.15***	−0.10**	−0.07*	0.29***	**0.73**	
8	Ability to defend	42%	–	–	0.11**	−0.09*	0.08*	−0.01	−0.04	0.40***	0.21**	–

*Cronbach's alpha in bold along the diagonal. *p < 0.05; **p < 0.01; ***p < 0.001.*

TABLE 2 | Main and interactive effects of exposure to bullying behaviors and ability to defend on symptoms of anxiety ($N = 737$).

Step	Variable	B	SE B	β	R^2	ΔR^2
1					0.04	
	Age	−0.00	0.00	−0.08		
	Seniority	−0.01	0.00	−0.03		
	Gender (reference category: "Male")	0.02	0.03	0.02		
	Leadership responsibility (reference category: "No")	−0.02	0.03	−0.02		
	Full-time employment (reference category: "No")	0.19	0.05	0.15***		
2					0.12	0.08
	Age	−0.00	0.00	−0.09*		
	Seniority	0.00	0.00	−0.01		
	Gender	0.02	0.03	0.04		
	Leadership responsibility	−0.01	0.03	−0.01		
	Full-time employment	0.17	0.05	0.13***		
	Bullying behaviors (Bullying)	0.25	0.04	0.24***		
	Ability to defend (AtD)	0.08	0.03	0.10*		
3					0.13	0.01*
	Age	−0.00	0.00	0.08*		
	Seniority	−0.00	0.00	0.01		
	Gender	0.03	0.03	0.04		
	Leadership responsibility	−0.00	0.03	0.04		
	Full-time employment	0.17	0.05	0.13***		
	Bullying	0.41	0.09	0.39***		
	AtD	0.07	0.03	0.08*		
	Interaction term: Bullying*AtD	−0.19	0.10	−0.16*		

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.*

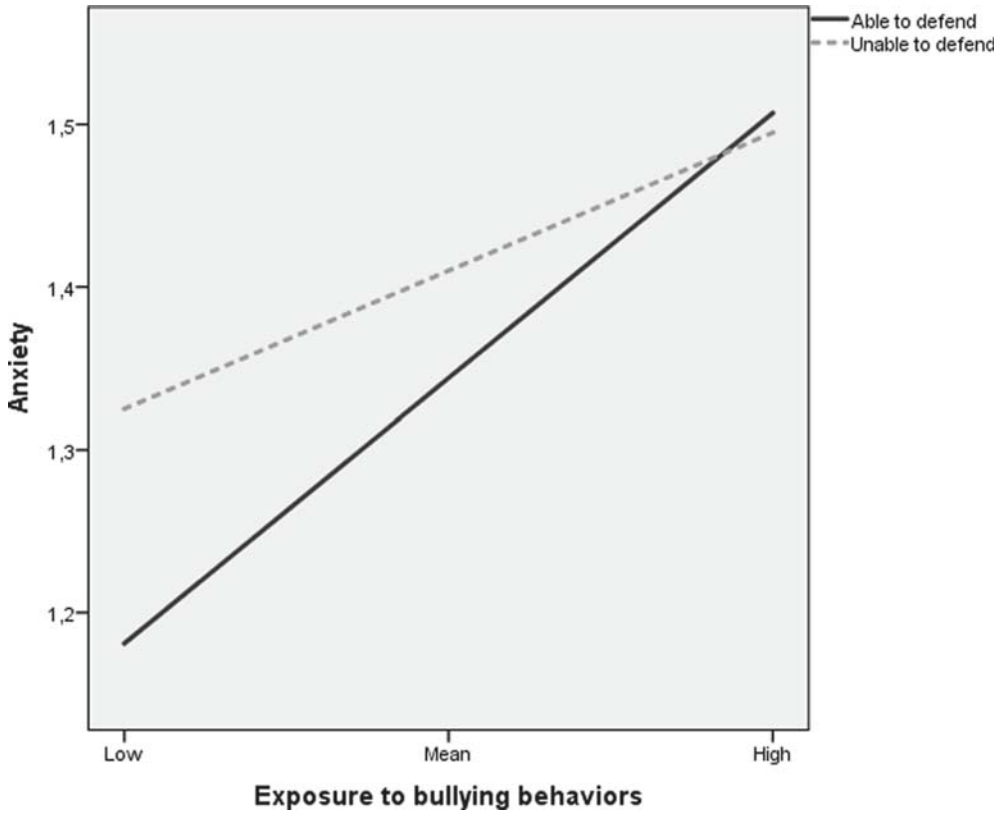

FIGURE 1 | Interactive effect of exposure to bullying behaviors and ability to defend on symptoms of anxiety.

An implication of situational incongruence is that the effects of exposure to bullying may be more prominent when interpreted against a backdrop of a positive view of oneself and the world (Nielsen et al., 2008). Consequently, for a target with an overall pervasive and enduring feeling of confidence displayed through a perception of being able to defend oneself, long-lasting and systematic exposure to severe forms of bullying may have especially negative effects because it is unanticipated and creates a pervasive feeling of dissonance in the target. Thus, being repeatedly exposed to bullying over a long period of time may result in an incongruity between the self-perception of persons able to defend themselves and how they feel they are treated by the bullies (Nielsen et al., 2008). As we need consistency in our conceptual system, such unresolved incongruence may be experienced as deeply shattering and may consequently result in psychological distress (Janoff-Bulman, 1992; Mikkelsen and Einarsen, 2002a).

The findings of a reverse buffering effect of ability to defend may also be explained by the very nature of workplace bullying as a stressor (Zapf and Einarsen, 2005). Unlike exposure to other stressors encountered at work such as job demands and role stressors, "...the aggressive behavior experienced by targets of bullying is likely to thwart the satisfaction of fundamental psychological and relational needs (e.g., sense of belonging and trust in others) and thereby inflict severe psychological, emotional, and even physical reactions" (Hauge et al., 2010, p. 427). Bullying is a particularly strong stressor that by its very nature is difficult to defend against, particularly at the workplace where fleeing or avoiding the situation is not really an option, at least in the short run. In addition, bullying is a one-sided event where the target per definition is unable to control the situation. In line with the findings of the current study, this may imply that perceived ability to defend may have a ceiling effect, being beneficial under exposure to milder forms of bullying (e.g., more like incivility, see Hershcovis et al., 2017), whereas the ability to defend does not protect targeted employees in cases of systematic harassment.

Strengths and Limitations

In terms of strengths, the present study is based on a large and randomly selected sample of Norwegian employees. Both exposure to workplace bullying and anxiety were assessed with well-established and psychometrically sound measurement instruments. With 32% response, the overall response rate was lower than the average rate of 52% which has been established for survey research (Baruch and Holtom, 2008). Yet, while response rate has important implication for the external validity (i.e., generalization) of studies, it can be questioned whether it has any significant impact on the internal validity of a study (Schalm and Kelloway, 2001; Nielsen and Knardahl, 2016).

As all cross-sectional questionnaire surveys, our study does not account for the causal relationships between the

study variables. Although we have investigated the theoretical assumption that the ability to defend moderates the relationship between exposure to workplace bullying behaviors as predictor variable and anxiety as outcome variable (see Reknes et al., 2014 for evidence), other kinds of relationships are also likely. For instance, some prospective studies have shown that the association between bullying and mental health is bidirectional (Nielsen and Einarsen, 2012; Nielsen et al., 2014; Verkuil et al., 2015). To provide indications of causality, longitudinal studies on bullying, ability to defend, and anxiety are needed.

All data were collected using self-report questionnaires, something which could hamper the internal validity of the findings. For instance, there is the possibility of common method variance and response set tendencies (Spector, 2006). Social desirability may be a likely form of response set. Social desirability is a form of response bias where the respondents answer questions in a manner that will be viewed favorably by others. It can either be over-reporting "good behavior" or under-reporting "bad," or undesirable behavior (Phillips and Clancy, 1972). Relying on self-report methodology may be especially problematic with regard to assessing workplace bullying, ability to defend, and anxiety due to feelings of shame and guilt among respondents (Hauge et al., 2009). Yet, one may also argue that self-report is the only valid measure of these particular individual and psychological states.

The respondents' ability to defend was measured with a single item developed specifically for this study. The use of single-item measures is often discouraged from a psychometric point of view as such measures may suffer from reliability and validity issues (Nielsen et al., 2013a). This rigorous view of single-item measures has recently been challenged (Wanous and Reichers, 1996; Wanous et al., 1997; Gardner et al., 1998). As highlighted by Olsen et al. (2012), single-item measures can be reliable, as estimated by test-retest correlations (Littman et al., 2006), correlate strongly with multiple-item scales (Wanous et al., 1997), and can predict outcomes effectively (e.g., Nagy, 2002). While single-item measures have limitations, they do also have clear advantages, such as cost-efficiency, greater face validity, and the increased willingness of respondents to take time to complete the questionnaire when the number of items is reduced (Olsen et al., 2012). The single-item method used in this study was found to correlated adequately with the most frequently used indicators of workplace bullying (self-labeling method and behavioral checklist) and do thereby seem to be a valid and reliable indicator of ability to defend. Nonetheless, to further elucidate the impact of ability to defend, a scale instrument should be developed for future studies.

CONCLUSION, IMPLICATIONS, AND FURTHER RESEARCH

The present study showed that the perceived ability to defend oneself against workplace bullying behavior is only protective against symptoms of anxiety in cases of low exposure. This protective effect diminishes in cases where the bullying is more systematic and severe. Specifically, in cases of high exposure to bullying, there was a stronger increase in anxiety among employees able to defend themselves than among those felt unable to defend. As for relative levels of anxiety, the findings suggest that in cases of high exposure to bullying, targets report equal levels of anxiety irrespectively of their ability to defend. Hence, adhering to some previous studies (Vie et al., 2011; Nielsen et al., 2008; Reknes et al., 2016), our study further demonstrate that bullying is a detrimental experience for all those exposed, irrespective of their personal coping resources.

This finding has several important implications, be it for theory, for practice and for methodology. With regard to theory, it has been argued that although all interaction types have the potential for advancing theory, "the buffering interactions hold the greatest potential because they are more likely to challenge existing perspectives" (Andersson et al., 2014, p. 1068). In line with this claim, the reverse buffering interaction established in this study questions a central assumption in stress theory, namely that personal dispositions and personal resources will act to protect individuals against the potential negative impact of stressors. If our findings can be validated in upcoming research, preferably with designs that allows for causal interpretation (see Ilies et al., 2011; Reknes et al., 2016), stress theories must take into consideration that the protective power of personal resources may be dependent upon type of stressor rather than solely assuming that personal factors buffers the negative impact of all stressors. Alternatively, the established reverse buffering effect may suggest that personal resources have some sort of ceiling effect with regard to bullying in that they are only beneficial under low exposure.

The results of this study may also have important implications for the understanding of workplace bullying as a phenomenon. As most definitions highlight power imbalance as a main characteristic, the results of this study suggest that bullying may be detrimental even when targets perceives to have the ability to defend themselves against the mistreatment. Hence, our findings indicate that it is the very magnitude and frequency of the exposure that constitutes the menace rather than the perceived power differences between target and perpetrator. Alternatively, it may be that the power imbalance is actually manifested in the very exposure, and that this imbalance has a more profound impact on the target as compared to the subjective perception of being able to defend oneself. Nonetheless, as our study only represents a single contribution to the field, further research is needed in order to comprehend the impact of bullying on its targets.

As for methodology, the present study indicates that behavioral checklists such as the NAQ, are valid measures of workplace bullying even if not explicitly measuring all aspects of the theoretical definition, in this case not explicitly measuring the ability to defend oneself in the actual situation. With regard to practice, knowledge about factors that protects workers against workplace bullying is highly important for both managers, consultants, counselors and medical personnel. The relationships between bullying, ability to defend, and anxiety found in this study provide organizations and practitioners with important information about how to prevent and handle bullying. It has been proposed that organizations could use personality

testing to identify potential targets and thereby to focus anti-victimization interventions at the identified individuals and their workplaces (Bowling et al., 2010). In light of previous studies which have found that bullying impacts all, irrespective of their personality characteristics (Nielsen et al., 2008; Reknes et al., 2016), our findings indicate that individual capacities have little protective impact with regard to bullying. Hence, it can be discussed whether testing and identifying individuals with specific personality characteristics have any merit.

As being exposed to bullying may be experienced as particularly devastating and harmful by the presumably robust employee due to incongruence and dissonance, organizations and employers must actively intervene in the early stages of the bullying process rather than believing that the said targeted worker should be able to deal with the exposure him-/herself. Previous research have shown that organizational factors, such as climate for conflict management, may be especially valuable with regard to managing workplace bullying (Einarsen et al., 2016). Consequently, focusing on primary interventions, such as building a strong psychosocial safety climate may be the most effective way to prevent workplace bullying from occurring and harming employees (Bond et al., 2010; Law et al., 2011). However, in cases where bullying does occur, organizations must have effective, and preferably pre-defined, secondary and tertiary intervention strategies in place.

ETHICS STATEMENT

The study was approved by the Regional Committees for Medical and Health Research Ethics (REK) for eastern Norway and was conducted in accordance with the World Medical Association Declaration of Helsinki. All study participants provided were provided information that informed consent was given by answering the questionnaire. Data were de-identified for analyses. The consent procedure was approved by the REK.

AUTHOR CONTRIBUTIONS

All authors contributed to the development of this study and the writing of the manuscript. MN was responsible for the data analyses and the first draft of the paper. JG, DJ, and SE contributed to the writing and provided quality checks of data analyses.

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II

Original article

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Negative social acts and pain: evidence of a workplace bullying and 5-HTT genotype interaction

by Jacobsen DP, Nielsen MB, Einarsen S, Gjerstad J

This paper examines the interaction between bullying and 5-HTT genotype with regard to pain intensity in the general working population (N=987). The data revealed that the association between bullying and pain is moderated by a genetic variation in the 5-HTT gene, and that the association between negative social acts and health in vulnerable individuals may be far more potent than previously reported.

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Key terms: 5-HTT; 5-HTT genotype; 5-HTTLPR; bullying; negative social act; pain; polymorphism; psychosocial; rs23351; serotonin transporter; SLC6A; workplace bullying

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Please note that there is additional material available belonging to this article on the *Scandinavian Journal of Work, Environment & Health* -website.

Original article

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Negative social acts and pain: evidence of a workplace bullying and 5-HTT genotype interaction

by Daniel Pitz Jacobsen, MSc,[1] Morten Birkeland Nielsen, PhD,[1,2] Ståle Einarsen, PhD,[3] Johannes Gjerstad, PhD [1,2,3]

Jacobsen DP, Nielsen MB, Einarden S, Gjerstad J. Negative social acts and pain: evidence of a workplace bullying and 5-HTT genotype interaction. *Scand J Work Environ Health.* 2018:44(3):283–290. doi:10.5271/sjweh.3704

Objectives Long-term exposure to systematic negative acts at work, usually labeled workplace bullying, is a prevalent problem at many workplaces. The adverse effects of such exposure may range from psychological symptoms, such as depression and anxiety to somatic ailments like cardiovascular disease and musculoskeletal complaints. In this study, we examined the relationships among exposure to negative acts, genetic variability in the 5-HTT gene *SLC6A4* and pain.

Methods The study was based on a nationally representative survey of 987 Norwegian employees drawn from the Norwegian Central Employee Register by Statistics Norway. Exposure to bullying in the workplace was measured with the 9-item version of the Negative Acts Questionnaire – Revised (NAQ-R) inventory. Pain was rated using an 11-point (0–10) numeric rating scale (NRS). Genotyping with regard to *SLC6A4* was carried out using a combination of gel-electrophoresis and TaqMan assay.

Results The data revealed a significant interaction between exposure to negative acts and the *SLC6A4* genotype with regard to pain (linear regression with 5000 resamples; age, sex, tobacco use and education were included as covariates). The relationship between negative acts and pain intensity was significantly stronger for subjects with the L_AL_A genotype than for subjects with the $SL_A/L_AL_G/SL_G$ genotype. No significant difference between subjects with the L_AL_A genotype and SS genotype was observed.

Conclusions Our data demonstrated that the relationship between bullying and pain was modified by the 5-HTT genotype, ie, genetic variation in *SLC6A4*. The association between negative acts and health among vulnerable individuals appeared more potent than previously reported.

Key terms polymorphism; psychosocial; rs23351; serotonin transporter; SLC6A; 5-HTTLPR.

Exposure to systematic negative social acts at work, usually labeled workplace bullying, is a prevalent issue in contemporary working life, affecting approximately 15% of adults globally (1). Several lines of evidence demonstrate that exposure to such bullying is a major predictor of impaired health and well-being among those targeted (2, 3). The adverse effects of bullying is well documented and range from psychological symptoms, such as depression and anxiety (4, 5), to somatic ailments like cardiovascular disease (6) and musculoskeletal complaints (7). Exposure to bullying is also associated with increased risk of sickness absence (8) and disability retirement (9).

While exposure to bullying in the workplace is a risk factor for pain (10, 11), pain may also be determined by the individuals psychological profile and genetic susceptibility (12). Interestingly, as much as 60% (range 25–60%) of the variance in experimental pain may be explained by genetic variability (13). Thus, pain perception is subject to large variation between individuals. Earlier studies suggest that pain in an experimental setting may be associated with genetic variability important for serotonin (5-HT) signaling (14, 15).

One genetic variant that may be important for 5-HT signaling is the 22-base-pair variable number tandem repeat (5-HTTLPR) in the promoter of the SLC6A4

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gene encoding the serotonin transporter (5-HTT). Two common allelic variants have been described, a short (S) allele of 14 repeats and a long (L) allele of 16 repeats (16). The short allele leads to decreased 5-HTT expression (17). In addition, there is a single nucleotide polymorphism (SNP) in the promoter region of SLC6A4, which also affects the rate of transcription (18). This A to G substitution is in strong linkage disequilibrium with the length polymorphism of the promoter, where the G allele, associated with lower expression, almost always coincides with the long allele (19).

The most important transmitters in the pain pathways may include the excitatory signaling molecule glutamate and the inhibitory modulator GABA. In the central nervous system, 5-HT is a modulator of both glutamatergic and GABAergic neurotransmission (20, 21). Hence, polymorphisms influencing the efficacy of 5-HTT – responsible for 5-HT reuptake into the synaptic boutons – may affect signaling in the pain pathways and nociceptive processing in the brain. Based on the presumed transcription rates from low to high (15), the Caucasian population can be divided in three groups; low (SS), medium ($SL_G/L_AL_G/SL_A$) and high (L_AL_A) expression. Individuals with low, medium and high expression may have different phenotypes.

For example, previous data have suggested that pain evoked by colorectal distention in individuals with SLC6A4 low-transcription-genotype induces an increased activation of brain areas involved in emotion-regulation (22). Moreover, people with SLC6A4 low-transcription-genotype may be associated with anxiety and negative affect (23). On the other hand, individuals with SLC6A4 high-transcription-genotype seem to report more pain evoked by thermal stimuli (24).

Recent data show that exposure to bullying at the workplace is associated with increased distress and somatic health complaints (25). Less is known about conditional factors that govern the health consequences of bullying. However, based on the possible link between bullying, *SLC6A4* genotype and pain, we hypothesized that the effect of bullying on pain may be modified by genetic variation in *SLC6A4*. In the present study, we demonstrate that pain in the working population is associated with a bullying and *SLC6A4* genotype interaction.

Methods

Subjects

This study is based on a probability sampled survey of the Norwegian working force. A random sample of 5000 employees was drawn from The Norwegian Central Employee Register by Statistics Norway. The Norwegian Central Employee Register is the official register of all Norwegian employees, as reported by employers. Sampling criteria were adults aged 18–60 years employed in a Norwegian enterprise. Questionnaires were distributed through the Norwegian Postal Service during the spring 2015. Subjects who gave consent were also sent saliva collection kits. Altogether, 987 subjects who had satisfactorily completed the questionnaire and given a saliva sample were included in this study. The survey was approved by the Regional Committee for Medical Research Ethics for Eastern Norway. Responses were treated anonymously, and informed consent was given by the respondents.

Instruments

Exposure to bullying behaviors in the workplace was measured with the 9-item version of the Negative Acts Questionnaire – Revised (NAQ-R) inventory (26). NAQ-R describes negative and unwanted behaviors that may be perceived as bullying if occurring on a regular basis. The NAQ-R contained items referring to both direct (eg, openly attacking the victim) and indirect (eg, social isolation, slander) behaviors. It also contained items referring to personal as well as work-related forms of bullying. For each item, the respondents were asked how often they had been exposed to the behavior at their present worksite during the last six months. Response categories range from 1–5 ("never", "now and then", "monthly", "weekly" and "daily").

To assess pain, subjects were asked to rate their mean general pain intensity throughout the last week using an 11 point (0–10) numeric rating scale (NRS) with endpoints "no pain" and "worst possible pain".

Genotyping

Collection of saliva and extraction of genomic DNA was done using OrageneRNA sample collection kit (DNA Genotech Inc. Kanata, Ontario, Canada) according to the manufacturer's instructions. Genotyping with regard to *SLC6A4* tandem repeat length in the promoter (short: S versus long: L), and genotyping with regard to the SNP rs23351 (A versus G) were performed.

To determine the length (S versus L) of the polymorphic promoter region of *SLC6A4*, the DNA sequence was first amplified by polymerase chain reaction (PCR) and then separated by gel electrophoresis. PCR was carried out in a total volume of 25 µl containing ~60 ng of genomic template, 6.25 pmol of each primer and 1×Taq DNA Polymerase Master Mix (VWR international, Dublin, Ireland). The forward primer sequence was 5' –GGCGT TGCCG CTCTG AATGC- 3' and the reverse primer sequence was 5' –GAGGG ACTGA GCTGG ACAAC CAC- 3' (DNA technology A/S, Riss-

kov, Denmark). As previously described (27), samples were amplified on a Perkin Elmer GeneAmp PCR 2400 system following an initial denaturing step for 3 minutes at 95 °C. The amplification consisted of 40 cycles including denaturing at 95 °C for 40 seconds, annealing at 60 °C for 20 seconds and elongation at 72 °C for 80 seconds. The PCR yielded a long (529 bp) and a shorter (486 bp) fragment. After four hours separation at 100 V on a 2.5% agarose gel (MetaPhor Agarose, Lonza cologne GmbH, Cologne, Germany), GelRed dye was added and the fragments were visualized by UV light (Biotium Inc, California, USA). A PCR 100 bp low ladder (Sigma-Aldrich CO, St. Louis, Mo, USA) was used to determine the length of the fragments.

The SNP genotyping with regard to rs23351 (A versus G) was carried out using custom TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA, USA). Approximately 10 ng genomic DNA was amplified in a 5 µl reaction mixture in a 384-well plate containing 1× TaqMan genotyping master mix (Applied Biosystems) and 1× assay mix, the latter containing the respective primers and probes. The probes were labelled with the reporter dye FAM or VIC to distinguish between the two alleles. Approximately 10% of the samples were re-genotyped and the concordance rate was 100%.

Statistical analysis

Exposure to bullying was calculated using the mean-score of the 9 items in the NAQ-R inventory. To explore the hypotheses about main and moderating effects, we conducted a hierarchical regression analysis to test for linear associations between exposure to bullying behaviors and experienced pain, as well as the interactive effects of exposure to bullying and *SLC6A4* genotype (three allele model), with regard to pain. Deviation from the Hardy-Weinberg equilibrium was tested by the Chi-squared test. In order to examine the modifying role of the *SLC6A4* genotype, we followed the recommendations for interaction analyses provided by Baron and Kenny (28). The *SLC6A4* genotype was included as a categorical variable using the L_AL_A genotype as a reference group. The interaction analysis was conducted in two steps. Control variables, exposure to bullying and the *SLC6A4* genotype were entered as predictors in the first step, whereas the interaction term (exposure to bullying×*SLC6A4*) was entered in the second step. A significant interaction term and a significant increase in explained variance (R^2) in the second step were considered as an interaction effect.

As the scores on the NAQ (skewness: 4.18; kurtosis: 26.85) were non-normally distributed, all analyses were conducted using bootstrapping (5000 resamples). The bootstrap method has the advantage that it does not need to meet the assumptions of normality, equal variances, and homoscedasticity that are required in ordinary regression analyses (29). Multicollinearity was not an issue in the current study [variance inflation factor (VIF)=1.01, with cutoff set at VIF=10). Statistical analyses were conducted with Stata 14 (StataCorp, College Station, TX, USA). The level of significance was set to $P<0.05$.

Results

The data showed that 551 of the 987 subjects (56%) included in this study experienced negative acts, ie, NAQ >1 at the workplace during the last six months. Mean NAQ and NRS scores were similar for men and women (NAQ: 1.19 and NRS: 2.52). Genotype frequencies of SS, SL_G, L_AL_G, SL_A and L_AL_A were 18.2%, 7.2%,

Table 1. Characteristics of the subjects grouped by genotype: SS, SLG/LALG/SLA and LALA. [SEM=standard error of the mean; VAS= visual analog scale; NAQ= Negative Acts Questionnaire.]

	SS				SLG/LALG/SLA				LALA				Sum
	N	%	Mean	SEM	N	%	Mean	SEM	N	%	Mean	SEM	
Subjects	180	18.2			555	56.2			252	25.5			987
VAS			2.38	0.16			2.66	0.01			2.29	0.13	
NAQ			1.15	0.02			1.20	0.01			1.19	0.02	
Age			45.08	0.78			45.07	0.43			44.35	0.62	
Male	92	51.1			256	46.1			117	46.4			
Female	88	48.9			299	53.9			135	53.6			
Tobacco	41	22.7			104	18.7			63	25.0			
Education													
Secondary school or less	13	7.2			49	8.8			22	8.7			
High school	55	30.6			163	29.4			75	29.8			
University ≤4 years	65	36.1			182	32.8			78	31.0			
University ≥4 years	47	36.1			161	29.0			77	30.6			

Table 2. Hierarchical regression with genotype LALA as reference (bootstrapping with 5000 resamples). The analyses were adjusted for the covariates age, sex, tobacco use and education. [SE=standard error; CI=confidence interval]

Pain	B	SE	P-value	95% CI
Step 1				
Age	0.009	0.007	0.198	-0.005–0.023
Sex	0.558	0.139	0.000	0.287–0.830
Tobacco use	0.470	0.179	0.009	0.119–0.820
Education				
High school	-0.208	0.295	0.482	-0.787–0.371
University <4 years	-0.809	0.283	0.004	-1.364– -0.255
University >4 years	-1.178	0.286	0.000	-1.738– -0.618
SLC6A4				
SS	0.145	0.201	0.471	-0.249–0.539
SLG LALG SLA	0.376	0.158	0.017	0.067–0.686
NAQ9	0.957	0.259	0.000	0.450–1.464
Step 2				
Age	0.009	0.007	0.170	-0.004–0.023
Sex	0.572	0.139	0.000	0.301–0.844
Tobacco use	0.494	0.178	0.005	0.145–0.843
Education				
High school	-0.182	0.297	0.539	-0.763–0.399
University <4 years	-0.791	0.285	0.005	-1.349– -0.233
University >4 years	-1.155	0.288	0.000	-1.719– -0.591
SLC6A4				
SS	0.563	0.851	0.508	-1.106–2.232
SLG LALG SLA	1.953	0.636	0.002	0.706–3.199
NAQ9	1.768	0.431	0.000	0.924–2.612
SLC6A4 x NAQ				
SS	-0.337	0.734	0.646	-1.776–1.101
SLG LALG SLA	-1.320	0.540	0.015	-2.379– -0.261

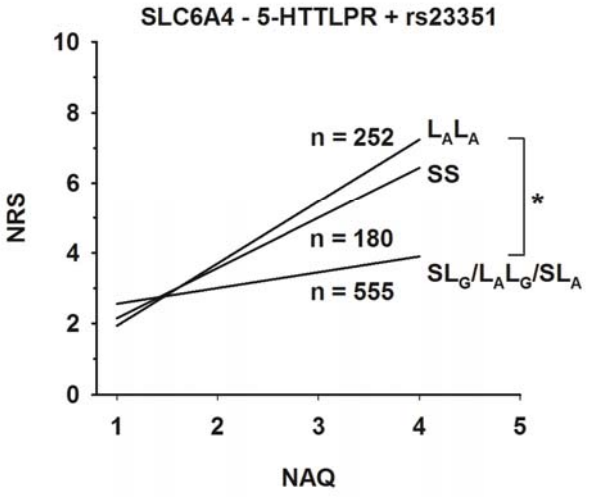

Figure 1. The relationship between negative acts and pain intensity (NRS), after correction for age, sex, tobacco use and education. Subjects were divided into groups based on SLC6A4 genotype: SS, SLG/LALG/SLA and LALA (used as reference for the regression analysis). *P<0.05. [NAQ=Negative Acts Questionnaire; NRS=numeric rating scale.]

6.8%, 41.2% and 25.5%, respectively. No deviation from the Hardy-Weinberg equilibrium was observed. The characteristics of the subjects are presented in table 1.

Findings from the hierarchical regression analyses of linear associations and interaction effects are presented in table 2. In the first step, exposure to bullying was significantly positively associated with pain. The $SL_G/L_AL_G/SL_A$ genotype, but not the SS genotype, reported significantly higher pain than the L_AL_A genotype reference group. Gender, tobacco use, and educational level, but not age, were also significantly related to pain experience. The model was significant (Wald X^2=81.16; P<0.001) and the predictor variables explained 8.36% of the variance in pain experience.

The interaction term (exposure to bullying×*SLC6A4*) was entered in the second step of the analysis. The findings demonstrated a significant interaction between exposure to negative acts and 5-HTT genotype with L_AL_A genotype used as reference with regard to pain experience. The statistical model with the interaction term explained 9.15% of the variance in pain. The model with the interaction term was also significant (Wald X^2=97.83; P<0.001).

The relationship between reported negative acts and pain intensity was significantly stronger for subjects with the L_AL_A genotype than for subjects with $SL_G/L_AL_G/SL_A$ genotype (figure 1). No significant difference between subjects with the L_AL_A genotype and subjects with the SS genotype was observed.

Similar hierarchical regression analyses were performed with the 5-HTTLPR length polymorphism and the SNP genotype separately. The data showed that subjects with LL or AA genotypes also had significantly stronger relationships between negative acts and pain intensity than other subjects (supplementary tables A and B, figures A and B, www.sjweh.fi/show_abstract.php?abstract_id=3703).

Discussion

In accordance with previous findings, our data showed that experiencing negative acts in the workplace is positively correlated with pain intensity (10, 11, 30). The mechanisms behind this association are unknown but may involve psychological distress as an intermediate factor. Previous data suggest that exposure to bullying behaviors results in symptoms such as depression and anxiety (31, 32), which in turn may be associated with pain (33–35).

Several lines of evidence suggest that the short 5-HTTLPR allele may be associated with increased sensitivity to stress (36). Moreover, previous data suggest that the influence of life stress on depression may be moderated by genetic variability in *SLC6A4* (37–39). However, this gene-environment interaction may be debated (40–42).

Our data showed that the association between negative acts and pain may be moderated by genetic variation within the promotor region of *SLC6A4*. Interestingly, subjects with the high expression L_AL_A genotype reported more pain than those with the medium expression SL_G/L_AL_G/SL_A genotype when exposed to systematic bullying behaviors. However, there was no difference between subject with the L_AL_A genotype and those with the SS genotypes. In accordance with earlier data on experimental pain (15), the L_AL_A genotype was associated with the highest pain ratings in the present survey.

A higher frequency of the SS genotype has been observed among patients with fibromyalgia or idiopathic trigeminal neuralgia than healthy controls (43, 44). Moreover, subjects with the SS and SL_G genotypes may also report increased intensity of pain following topical alcohol disinfection of epidermal abrasions (45). In addition, enhanced pain catastrophizing has been reported in S-carriers, suggesting that the low and medium expression (SS / SL_G / L_AL_G / SL_A) genotypes might be a risk factor for emotional pain (46, 47).

On the other hand, animal experiments have demonstrated that knockout mice completely lacking 5-HTT show reduced thermal hyperalgesia compared to wild type mice (48, 49). Moreover, sensory testing of humans show that thermal or electrical noxious stimuli induces increased sensory pain in individuals with the high expression (L_AL_A) genotype (15, 24). Thus, the relationship between the expression of 5-HTT and subjective health complaints may not necessarily be linear.

Hence, although previous data have demonstrated enhanced emotional responses or increased pain catastrophizing in S-carriers (46, 47), testing of humans in the lab shows that individuals with L_AL_A have the strongest pain response to sensory stimuli (15, 24). Therefore, subjects with the SS and L_AL_A genotype are not very different. The SS genotype may be associated with increased emotional pain, whereas the L_AL_A genotype seems to be associated with increased sensory pain. This may explain the result that no significant difference in pain score was observed between subjects with SS versus L_AL_A. In accordance with our earlier observations (15), the present data suggest a u-shaped relationship between presumed *SLC6A4* transcriptional rate and pain intensity.

Anyway, the rate of transcription is dependent on both the 5-HTTLPR and the SNP rs23351 in the promoter region of *SLC6A4*. Therefore, our analyses based on only length polymorphism or alternatively only the SNP genotype resulted in lower explained variance than the model that was based on a combination of 5-HTTLPR and rs23351. Thus, combining these polymorphisms – which are in strong LD – produced a better statistical model. Hence, in accordance with previous observations (14, 15, 18, 50), the present data show that the model based on SS versus SL_G/L_AL_G/SL_A versus L_AL_A may be recommended.

Study limitations

The observed genotype frequencies were in accordance with previous findings (50). However, the overall response rate for the questionnaire survey was only 32%, and <20% of the invited participants returned the saliva samples. These numbers are both lower than the average response rate established for survey studies (51). Hence, we cannot be certain that the final sample is representative for the overall population or survey pool. Still, as response rate and representativity seems to have limited impact on the internal validity (52), the response rate may not be a problem with regard to the actual findings of this study. On the other hand, because measurement instruments for bullying and pain were self-report measures, the study could be influenced by bias such as response set tendencies and social desirability. In addition, a previous longitudinal study from Norway showed that dropout respondents reported significantly higher levels of exposure to bullying at baseline measurement (31). Therefore, non-responders could be more prone to have experienced negative social acts compared to responders.

Concluding remarks

In summary, our data demonstrated that the relationship between bullying and pain was modified by the 5-HTT genotype, ie, genetic variation in the promotor region of *SLC6A4*. Moreover, the present data showed that the effect of bullying on health and well-being among vulnerable individuals might be stronger than previously reported. We conclude that the effect of negative acts and pain is dependent on a gene-environment interaction.

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Conflicts of interest

The authors declare no conflicts of interest.

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