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A Novel Longitudinal Epigenome-Wide Study of Posttraumatic Growth

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Abstract

In this first epigenome-wide association study examining posttraumatic growth (PTG), a longitudinal approach assessing DNA methylation epigenome-wide pre- and post-trauma exposure in first-year paramedicine students was employed. The study identified two CpGs (cg09559117 and cg05351447) within the *PCDHA1*/*PCDHA2* and *PDZD* genes were significantly associated with PTG at the epigenome-wide threshold ($p < 9.42 \times 10^{-8}$). DNAm in 5 CpGs across known PTSD candidate genes *ANK3*, *DICER1*, *SKA2*, *IL12B* and *TPH1* were significantly associated with PTG after gene-wise Bonferroni correction. Pathway analysis revealed that genes associated with PTG at the suggestive level of significance ($p < 5 \times 10^{-5}$) were significantly overrepresented in the Adenosine triphosphate Binding Cassette (ABC) transporters pathway ($p = 2.72 \times 10^{-4}$). These results identify novel genes for PTG, improving our understanding of the neurobiological underpinnings of PTG.

Keywords: posttraumatic growth; posttraumatic stress disorder; stress; EWAS; DNA methylation

1. Introduction

Posttraumatic growth (PTG) describes both the process of positive psychological change resulting from exposure to extreme challenges, as well as the resulting improvements across varied domains of psychological functioning (Tedeschi & Calhoun, 2004). These domains include interpersonal relationships, perceptions of personal strength, appreciation for life, and spiritual and existential beliefs (Tominaga et al., 2020). PTG is common following trauma exposure and represents important psychological processes of the interaction of ongoing stress resulting from the exposure to a traumatic event and positive trajectories afterwards (Feder et al., 2008; Sattler et al., 2014). For example, Vietnam War veterans who had been prisoners of war reported positive outcomes resulting from their experience, including increased optimism, social support, and adaptive coping (Feder et

al., 2008). The capacity to grow and adapt can enable individuals to develop new skills and thrive following traumatic experiences.

The model of PTG conceptualises trauma as a challenge to an individual's core beliefs. The process of PTG is then the cognitive processing and resolution of this challenge and the eventual integration of this resolution into new beliefs (Calhoun & Tedeschi, 2014). In contrast, posttraumatic stress disorder (PTSD) is the ongoing conflict experienced by an individual who has not resolved the challenge to their beliefs that was presented by the traumatic experience. The relationship between exposure to trauma and negative sequelae is well established, with PTSD having a lifetime prevalence of between 0.5-9% in adult Western populations (American Psychiatric Association [APA], 2013; Bernhard et al., 2018). Epidemiological studies estimate that 8-12% of adults who experience a traumatic event develop PTSD (Breslau, 2009). Despite PTG being more common as a posttraumatic outcome (Feder et al., 2008; Sattler et al., 2014), PTSD has been the predominant focus of genomics research (Mehta et al., 2020).

The processes of PTG and PTSD are not mutually exclusive and have been shown to co-occur following trauma exposure (Laufer & Solomon, 2006; Powell et al., 2003). The nature of the relationship between the two outcomes has remained ambiguous, with studies suggesting a significant positive relationship between symptoms of PTSD and PTG (Taku et al., 2008), a significant negative relationship (Kılıç & Ulusoy, 2003), or no relationship at all (Ho et al., 2005). A meta-analysis of 42 papers within populations of varied backgrounds, ages, and trauma types found that a curvilinear model was a significantly stronger predictor of the relationship between PTG and PTSD symptoms (Shakespeare-Finch & Lurie-Beck, 2014). The relationship was affected by age at the time of exposure, with children fitting the curvilinear model more strongly than adults, and type of trauma. This meta-analysis represents one of the largest attempts at quantifying the relationship between PTSD and PTG. An approach that has only begun to emerge in recent years has involved the measurement of biological factors and underlying genetics as possible drivers of differences in posttraumatic outcomes, especially PTSD versus PTG.

Genetic factors have been well-established as contributing to the development of PTSD following trauma exposure (Broekman et al., 2007; Nievergelt et al., 2019). The effect of genetics on PTG, however, is comparatively under-researched (e.g., Dell'Osso et al., 2023). The gene-environment interaction (GxE) in a population of non-Hispanic African American parents exposed to a natural disaster was the first to include an assessment of PTG (Dunn et al., 2014). The study explored whether common variants of seven genes (*BDNF, CACNA1C, CRHR1, FKBP5, OXTR, RGS2*, and *SLC6A4*) modified the association between Hurricane Katrina exposure, PTSD, and PTG. A nominally significant association was found between a variation in *FKBP5* and PTG that did not survive correction for multiple testing (rs1306780, p=0.0113). Additionally, a significant association was found between a variant of the *RGS2* gene and PTG that did survive correction for multiple testing (rs4606; p=0.0044). This variant interacted with the severity of trauma exposure such that individuals with low levels of exposure showed PTG scores, and individuals with moderate or high levels of exposure showed increased levels of PTG. This *RGS2* variant had been shown to moderate the association between trauma severity and PTSD in a previous study, with decreased levels of PTSD symptom severity (Amstadter et al., 2009). The *RGS2* gene codes for a protein that regulates G-protein signalling and modulates neurotransmitter response, with different variants of this gene accelerating the deactivation of G-proteins at different rates (Kimple et al., 2009).

While the DNA code remains stable over the lifespan, epigenetic processes, such as DNA methylation (DNAm), are dynamic and affected by lived experiences. DNAm involves the addition of a methyl-chemical group to specific locations within the genome, which usually blocks access of transcription factors to the DNA, resulting in reduced

expression of that gene downstream (Jjingo et al., 2012). Trauma exposure has been associated with alterations in DNAm in epigenome-wide association studies (EWAS; Smith et al., 2011) as well as studies of specific candidate genes (Bick et al., 2012). An EWAS in Australian veterans identified DNAm at *DOCK2* associated with PTSD (Mehta et al., 2017), a gene previously associated with the formation of amyloid plaques in Alzheimer's disease (Cimino et al., 2013), thereby highlighting the importance of memory processes are important in post-trauma trajectories, this is a noteworthy discovery. A separate study examined DNAm before and after combat exposure in a cohort of male US military service members and found associations between PTSD and altered DNAm at *HEXDC* and *MADL1* genes, suggesting the involvement of immune pathways (Snijders et al., 2020).

Only one study has explored the association between PTG and DNAm. In a sample of 48 first-year paramedicine students, PTSD symptom severity, resilience, and PTG were associated with DNAm levels in the candidate genes of *FKBP5* and *NR3C1* (Miller et al., 2020). Specifically, hypomethylation at the CpG site cg07485685 within *FKBP5* was associated with increased PTSD symptom severity, while hypermethylation was associated with resilience. Differential DNAm in multiple sites across *FKBP5* and *NR3C1* was nominally associated with PTG, though these associations did not survive Bonferroni corrections.

In summary, the research on PTG thus far has only been cross-sectional in nature and has focussed on specific candidate genes. This novel study employs a longitudinal design to assess genome-wide changes in DNAm and their association with changes in PTG scores following exposure to a traumatic event. The aim of the study was to identify which genes and pathways are associated with PTG and compare the genes to those associated PTSD, to uncover the genetic etiology of PTG.

2. Methodology

2.1. Participants

Study details are reported in detail elsewhere (Miller et al., 2020). Briefly, participants were 40 first-year undergraduate Australian university paramedicine students. Participants were assessed at baseline during their first semester of classes (timepoint 1) and again 12 months later after completing field placement (timepoint 2). All 40 participants reported exposure to a potentially traumatic event(s) as part of their fieldwork placement. The study was approved by the Queensland University of Technology (QUT) and the University of Southern Queensland University (USQ) Human Research Ethics Committee. All participants provided written informed consent.

2.2. Assessments

At both timepoints, participants reported demographic information, including age, sex, ethnicity, alcohol consumption, smoking, and drug use. At baseline (timepoint 1), participants reported whether they had ever experienced a traumatic event, a brief description, and an assessment of the severity and distress at the time. At timepoint 2, participants reported whether they had experienced a traumatic event during their placement and a description and ratings of severity and distress on a Likert scale from 0-9, with higher scores indicating high levels of perceived severity and distress. In addition, participants completed assessments of PTG and PTSD at both timepoints and provided DNA via a saliva sample collected in an Oragene kit (DNA Genotek, USA).

2.3. Posttraumatic Growth Inventory X

The Posttraumatic Growth Inventory X (PTGI-X; Tedeschi et al., 2017) consists of 25 items that assess how much positive psychological change has occurred as a result of

exposure to a traumatic event. The items range from 0 (*Not at all*) to 5 (*A very great degree*), with higher scores indicating a greater level of growth. The PTGI-X has shown high reliability in US (α = .97), Turkish (α = .96) and Japanese samples (α = .95) (Tedeschi et al., 2017). The current sample also showed strong reliability (α = .96).

2.4. Posttraumatic Stress Disorder Checklist for DSM-V

The Posttraumatic Stress Disorder Checklist for Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-V) (PCL-5; Weathers et al., 2013) is a 20-item measure of PTSD symptom severity, with responses ranging from 0 (*not at all*) to 4 (*extremely*). Higher scores represent more severe symptoms. The measure can be interpreted by the overall summed score and interpreted via four sub-scales that correspond to criterion B, C, D, and E of PTSD in the DSM-V. The PCL-5 has displayed strong reliability and validity in US trauma-exposed student populations (Blevins et al., 2015). The current sample had strong reliability overall (α = .94) and within the subscales (ranging between α =.74 and α =.89).

2.5. Experiments

All experimental procedures have previously been described (Miller et al., 2020). Briefly, the saliva samples were sent to the Australian Genome Research Facility and stored at -20ºC. DNA was extracted from saliva using the Qiagen kit and quality assessment was performed by resolution on a 0.8 % agarose gel at 130 V for 60 minutes. Samples were bisulphite converted using the Zymo EZ DNA Methylation kit and hybridised on the Illumina EPIC array (Wockner et al., 2014). DNA for one sample did not satisfy quality standards at timepoint 2 and was removed from all further analyses, leaving 39 individuals across both time points and a total of 78 samples.

2.6. Statistical Analysis and Power Calculations

Data were analysed using an established analysis pipeline comprising of custom statistical programs and scripts (Barfield et al., 2014; Mehta et al., 2011; Mehta et al., 2013) written in R and Linux. Raw beta values from EPIC Illumina arrays were exported into R for statistical analysis. The raw DNAm data was background and control-normalized using the Bioconductor MINFI package (1.4.0; Aryee et al., 2014). A detection *p*-value was calculated for all arrays, where *p*-value >0.05 indicates methylation that is not significantly different from background measurements. We used excluded probes with p-detection > 0.01 in 10% or more samples. Samples with probe detection call rates <95% as well as those with an average intensity value of either <50% of the experiment-wide sample mean or <2000 arbitrary units (AU) were excluded from further analysis. This resulted in a total of 864,424 probes for all subsequent analyses. Cell counts were analysed using the Middleton method (Middleton et al., 2021). We used generalised linear mixed effects models to model the changes in DNAm at two timepoints, which we then regressed against the phenotype of interest (scores on the PCL-5 and PTGI-X). We corrected for covariates of age, sex, body mass index/BMI, cell counts, smoking, alcohol, drug use, and medication status using the lme4 package in R. For the epigenome-wide analyses, the epigenome-wide threshold ($p < 9.42 \times 10^{-8}$) was used to identify significant sites (Mansell et al., 2019), and the suggestive threshold of significance ($p < 5 \times 10^{-5}$) was used to denote suggestive sites of relevance (Kandaswamy et al., 2021). For the candidate genes, multiple testing across the different outcomes was adjusted using a gene-wise Bonferroni correction for multiple results to report results of interest. The hypergeometric test was used to test for the enrichment to assess if the observation is indeed statistically significant, i.e., beyond what is expected by chance, and this was performed in R. For the pathway analysis, CpGs were first annotated to genes using the Illumina EPIC array Manifest file and then assessed via

the KEGG pathway analysis through the online WEB-based GEne SeT AnaLysis Toolkit/ WebGestalt interface (Liao et al., 2019) using a false discovery rate of 5% to account for multiple testing corrections.

Analysis of the psychological variables was performed in IBM SPSS Statistics tool version 28.0.1.0. Changes in the PTG and PTSD scores between the two timepoints was performed via paired t-tests using 1000 bootstraps. Correlations between the psychological variables was performed using the non-parametric Spearman correlations.

Within a longitudinal study design, the paired-test method employs each subject as their own control, thereby removing between-subjects variability and increasing statistical power. The within-person correlations ranged between $.92 < r < .96$, with an average Spearman correlation $r = .94$ (SD = .007). These values are significantly higher than observed in similar papers within monozygotic twins (Tsai & Bell, 2015). Using the EPIC array power calculator, over 70% of the CpG sites arrayed have more than 90% power to detect small to moderate changes in DNAm (3-6%). These estimates of power are conservative given the longitudinal study design and the high within-person correlation observed in the study. Therefore, this study is well-powered to detect the observed (3-6%) DNAm changes.

3. Results

A total of 39 first-year paramedicine students at two Australian universities were included in the study. Psychological data via online surveys and DNAm via saliva samples was measured at two time-points - before (T_1) and after (T_2) exposure to potentially traumatic events. Study demographics are provided in Table 1. The participants were predominantly females (61.5%), Caucasian (89.7%), and with a mean age [SD] of 23.44 [1.08] years. In the current study, PTG and PTSD symptom severity were not significantly correlated at T_1 (Spearman correlation $r = .252$, $p = 0.122$) or T_2 (Spearman correlation $r = .140$, $p = 0.402$). There was a significant decrease in PTG scores from T_1 and T_2 ($p = 0.032$). There was a significant decrease in the overall PTSD PCL-5 score from T_1 to T_2 ($p = 0.029$) which was mainly driven by change in the sub-scale assessing cluster D symptoms of negative alterations in cognition and mood ($p = 0.004$). All other sub-scales showed non-significant differences between T_1 and T_2 ($p > 0.05$).

Table 1. – Demographics of the 39 paramedicine students included in the study.

Demographics/trait	Minumum	Maximum	Mean [SE]/ N [%]
			Overall sample
Age (in years)	17	43	23.44 [1.080]
Sex - Male			15 [38.5%]
- Female			24 [61.5%]
Ethcinity			
- Caucasian			35 [89.7%]
- Asian			2 [5.1%]
- African American			1 [2.6%]
- Aboriginal/ Torres Strait Islander			1 [2.6%]
Body Mass index/BMI	17.1	36.2	24.88 [0.75]
Current alcohol use			28 [71.8%]
Current medication			11 [28.2%]
Current smoking			5 [12.8%]

Current drugs			1 [2.6%]
Baseline - at start of paramedicine course			
Posttraumatic growth Inventory Score	6	120	72.05 [4.74]
Appreciation of Life	0	5	3.48 [0.19]
Personal Strength	0	5	3.36 [0.19]
New Possibilities	0	5	2.80 [0.24]
Relating to Others.	0.43	4.86	2.82 [0.20]
Spiritual and existential change	0	4.83	2.33 [0.21]
PTSD Symptoms Score (PCL)	0	50	16.82 [2.28]
PCL cluster B score	0	18	3.56 [0.67]
PCL cluster C score	0	8	1.95 [0.36]
PCL cluster D score	0	21	6.26 [0.92]
PCL cluster E score	0	12	5.05 [0.66]
Posttraumatic growth Inventory Score	6	120	72.05 [4.74]
Follow-up - post trauma exposure			
Posttraumatic growth Inventory Score	10	114	64.14 [3.95]
Appreciation of Life	0.33	4.67	2.99 [0.17]
Personal Strength	0.25	4.75	3.12 [0.18]
New Possibilities	0	4.8	2.36 [0.20]
Relating to Others.	0.57	4.71	2.75 [0.17]
Spiritual and existential change	0.17	4.2	1.86 [0.19]
PTSD Symptoms Score (PCL)	0	50	12.83 [2.27]
PCL cluster B score	0	13	3 [0.59]
PCL cluster C score	0	8	1.37 [0.35]
PCL cluster D score	0	20	3.97 [0.85]
PCL cluster E score	0	15	4.49 [0.70]

3.1. Candidate Genes Analysis

This is the first epigenome-wide analyses of PTG therefore as a proof of principle genes previously associated with PTSD were first tested to ascertain if these were also associated with PTG. Specifically, changes in PTG from T_1 to T_2 were tested for their association with DNAm changes in 55 candidate genes previously associated with PTSD (Mehta D, 2017). Of the 3,811 CpGs across 53 of the PTSD genes present in this dataset, 236 CpGs across 47 genes were nominally associated with changes in PTG scores ($p < 0.05$). Of these, 5 CpGs across five genes remained significant after a gene-wise Bonferroni correction, this is significantly greater than expected by chance alone (enrichment p-value = 0.003, Table 2). The genes included: ankyrin3 (*ANK3)*, dicer 1, ribonuclease III (*DICER1)*, spindle and kinetochore associated complex subunit 2 *(SKA2)*, interleukin 12B (*IL12B)* and tryptophan hydroxylase 1 (*TPH1)*.

Table 2. PTSD Candidate genes in PTG with atleast 10 CpGs tested and one bonferroni significant CpG identied.

Candidate genes	No. of cpgs tested	No of CpGs with p ≦ 0.05	Survive Bonferroni (N)
HDAC4	503	36	NO
CACNA1C	298	23	NO
RORA	237	13	NO
ANK3	160	13	YES (1)
DOCK2	106	6	NO
NOS1AP	94	12	NO
NR3C1	89	6	NO
NLGN1	86	7	NO
BDNF	84	5	NO
SLC6A3	81	8	NO
WWC1	77	5	NO
CRHR1	69	5	NO
ANKRD55	58	3	NO
NR3C2	53	7	NO
COMT	47	5	NO
DICER1	57	7	YES (1)
FKBP5	45	4	NO
HEXDC	44	1	NO
CAMKMT	44	1	NO
CRHR2	41	5	NO
DRD2	41	3	NO
ADCYAP1	40	2	NO
PDE1A	40	3	NO
MAN2C1	37	1	NO
ADCYAP1R1	36	4	NO
OXTR	36	1	NO
CNR1	35	5	NO
PRTFDC1	35	4	NO
LY9	34	4	NO
TPH2	33	2	NO
SLC6A4	31	3	NO
FOS	26	3	NO
GABRA2	26	4	NO
SLC18A2	26	1	NO
ALOX12	24	3	NO
NPY	22	1	NO
HTR1A	21	3	NO
SKA2	21	1	YES (1)
IL12B	19	2	YES (1)
RGS2	18	2	NO
DBH	18	1	NO

AIM2	16	1	NO
OPRL1	40	3	NO
ZNF626	14	2	NO
GBP1	13	1	NO
PRR11	13	2	NO
TPH1	11	2	YES (1)
Total	2999	236	5

3.2. EWAS of PTG

A hypothesis-free epigenome-wide analysis was performed to identify changes in DNAm associated with changes in PTG across the two time-points (before/T_1 and after exposure to a traumatic event/T_2). Across the 845k CpGs assessed, two CpGs were significantly associated with changes in PTG between T_1 and T_2 even after stringent correction at the epigenome-wide level (Mansell et al., 2019). The significant sites included cg09559117 in *PCDHA1/PCDHA2* ($p = 9.28 \times 10^{-8}$) and cg05351447 in *PDZD8* ($p = 9.39 \times 10^{-8}$, Figure 1). When using the suggestive level of significance of $p < 5 \times 10^{-5}$ (Kandaswamy et al., 2021), 99 CpGs across 71 genes were associated with changes in PTG scores across the two time-points (Table 3).

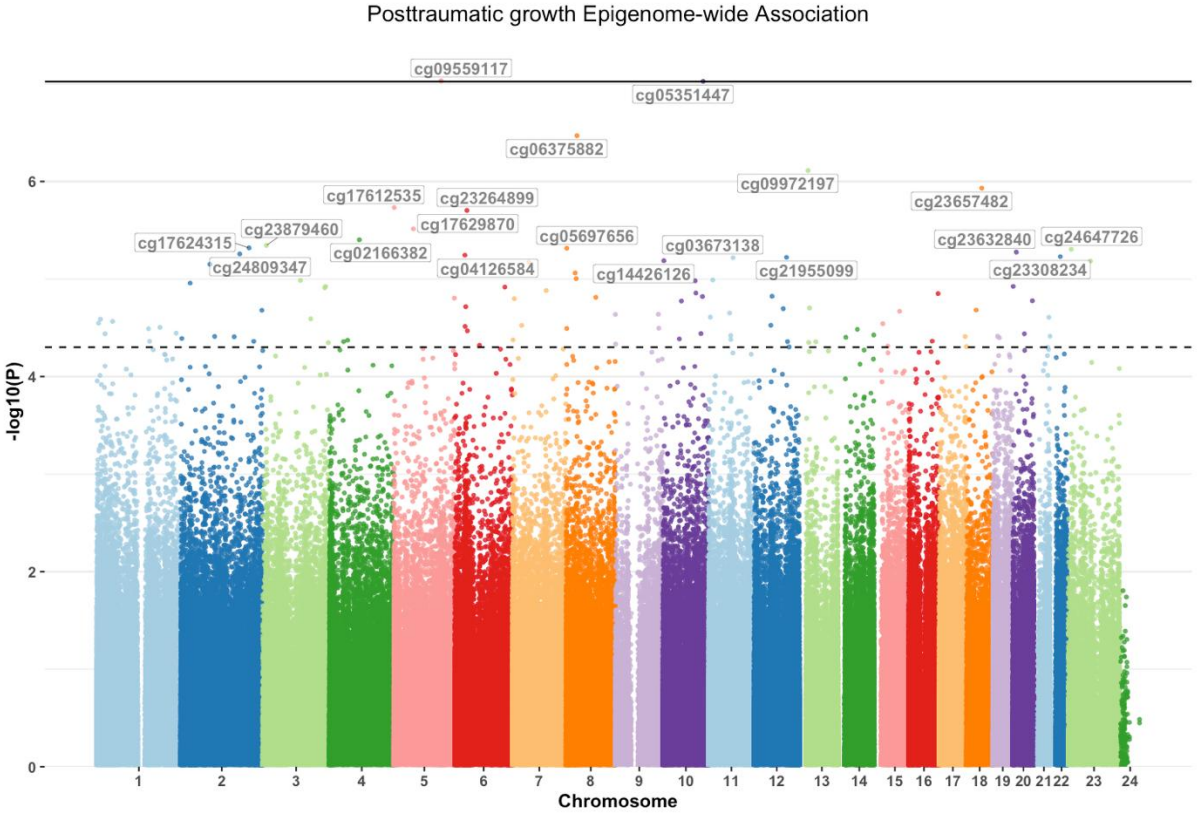

Figure 1. PTG associations: Manhattan plot showing epigenome wide DNAm associations for changes in PTG after trauma exposure.

Table 3. EWAS genes significantly associtatd with changes in PTG scores.

cpg	Pvalue PTG	Chromosome	Basepair	Gene Symbol
cg09559117	9.28E-08	5	140173855	PCDHA2;PCDHA1
cg05351447	9.39E-08	10	119120604	PDZD8

cg06375882	3.39E-07	8	32113523	NRG1
cg09972197	7.70E-07	13	26301550	ATP8A2
cg23657482	1.17E-06	18	45102036	
cg17612535	1.85E-06	5	932900	
cg23264899	1.98E-06	6	35765259	CLPS
cg17629870	3.06E-06	5	57756980	PLK2
cg02166382	3.96E-06	4	88496363	
cg23879460	4.52E-06	3	10806569	LOC285370
cg17624315	4.79E-06	2	202289200	TRAK2
cg05697656	4.83E-06	8	1897697	ARHGEF10
cg24647726	4.95E-06	X	11128608	HCCS
cg23632840	5.29E-06	20	10414722	C20orf94;MKKS
cg24809347	5.52E-06	2	174723194	
cg04126584	5.69E-06	6	29920309	
cg23308234	5.89E-06	22	29965207	NIPSNAP1
cg21955099	5.99E-06	12	96005661	
cg03673138	6.04E-06	11	72385963	PDE2A
cg14426126	6.49E-06	10	2394012	
cg17733714	6.55E-06	X	68114285	
cg10626169	6.73E-06	7	48319696	ABCA13
cg18825430	7.06E-06	2	86422958	IMMT
cg07572251	8.66E-06	8	26688088	ADRA1A
cg00739259	9.89E-06	8	29858411	
cg13332953	1.02E-05	11	12003759	DKK3
cg07479253	1.03E-05	3	111904892	SLC9A10
cg06789550	1.04E-05	10	95462915	C10orf4
cg16745960	1.10E-05	2	27549918	GTF3C2
cg02754380	1.19E-05	3	186369639	FETUB
cg01858394	1.19E-05	20	1277043	SNPH
cg14673315	1.21E-05	6	148336294	
cg00018767	1.23E-05	3	183693809	ABCC5
cg10714329	1.31E-05	7	100027122	MEPCE;ZCWPW1
cg14192396	1.39E-05	10	97416393	ALDH18A1
cg26384474	1.41E-05	16	86702325	
cg12831349	1.50E-05	12	52935087	
cg01399353	1.51E-05	10	117114665	ATRNL1
cg12533940	1.54E-05	8	88056685	CNBD1
cg13810079	1.57E-05	5	179484006	RNF130
cg00730549	1.59E-05	7	5430660	TNRC18
cg09887207	1.67E-05	20	58249281	PHACTR3
cg19492498	1.68E-05	10	54531460	MBL2
cg24478695	1.92E-05	6	32363167	BTNL2

cg03929569	1.98E-05	13	30689009	
cg06740227	2.01E-05	12	86229804	RASSF9
cg14263702	2.08E-05	18	28651637	DSC2;DSC2
cg01804434	2.09E-05	2	240456931	
cg08343397	2.14E-05	15	75340982	PPCDC
cg24078577	2.23E-05	11	62160859	ASRGL1
cg09039879	2.30E-05	9	127230734	
cg13793478	2.31E-05	9	109039	
cg17748470	2.46E-05	11	4969161	OR51A4
cg23107033	2.47E-05	21	44166176	PDE9A
cg27218767	2.56E-05	3	142442934	TRPC1
cg13085232	2.58E-05	1	10802080	CASZ1
cg26563242	2.72E-05	1	46797699	
cg27170935	2.83E-05	1	5221521	
cg03858387	2.87E-05	15	25199164	SNRPN;SNURF
cg05435504	2.98E-05	12	49251596	RND1
cg11908057	2.99E-05	7	27171154	HOXA4
cg01316659	3.06E-05	6	30418115	
cg27045794	3.13E-05	1	187412747	
cg08727313	3.19E-05	9	128734485	
cg06879681	3.21E-05	8	1900524	ARHGEF10
cg10228283	3.23E-05	1	153234387	LOR
cg03492327	3.28E-05	14	57273276	OTX2
cg00733115	3.39E-05	6	37105406	
cg04501323	3.58E-05	1	235267609	
cg17619701	3.62E-05	10	112610100	
cg21765224	3.64E-05	20	34359771	PHF20
cg21528040	3.64E-05	1	24195227	FUCA1
cg15895505	3.74E-05	14	105903354	MTA1
cg14669919	3.79E-05	11	65340482	FAM89B
cg04664999	3.85E-05	19	14185985	
cg00499599	3.85E-05	21	47706392	C21orf57;MCM3AP
cg17362661	3.87E-05	2	100210490	AFF3
cg12010144	3.88E-05	17	76733624	CYTH1
cg07568040	3.90E-05	2	158454401	ACVR1C
cg18887769	3.95E-05	14	22945181	
cg14251798	4.00E-05	19	19545333	MIR640;GATAD2A
cg06836148	4.07E-05	2	2957515	LINC01250
cg08920628	4.11E-05	10	48354911	ZNF488
cg20827116	4.17E-05	11	65627404	MUS81
cg11980004	4.20E-05	7	1571105	MAFK
cg24383710	4.23E-05	4	53916546	SCFD2

cg02645135	4.33E-05	16	69516238	
cg13056505	4.34E-05	1	156378014	C1orf61
cg22202891	4.35E-05	2	216001968	ABCA12
cg09468051	4.38E-05	4	41879262	
cg23053746	4.38E-05	12	98811404	
cg13290331	4.40E-05	13	49068807	RCBTB2
cg01660473	4.48E-05	13	28395757	
cg01243529	4.49E-05	3	194223220	
cg01183384	4.65E-05	9	716332	KANK1
cg02281539	4.78E-05	6	73273769	
cg20259534	4.88E-05	15	40453036	BUB1B
cg18456621	4.93E-05	17	80297270	
cg19359858	4.97E-05	12	103667687	C12orf42

Next, the biological pathways of the genes associated with PTG at the suggestive level of significance ($p < 5 \times 10^{-5}$) and those at a less stringent significance threshold ($p < 0.001$) was assessed using KEGG pathway database via the online WebGestalt interface (Liao et al., 2019). The genes (n=71) that were associated with PTG at $p < 5 \times 10^{-5}$ were significantly enriched in only the Adenosine triphosphate Binding Cassette (ABC) transporters pathway ($p = 2.72 \times 10^{-4}$). The genes (n=1,150) associated with PTG at $p < 0.001$ were significantly enriched in various pathways as shown in Table 4. The top pathways included Phospholipase D signalling, Axon guidance, EGFR tyrosine kinase inhibitor resistance, morphine addiction and dopaminergic synapse pathway.

Table 4. Biological pathways overrepresented among genes of CpGs associated with PTG at p<5e-5 and p < 0.001.

Pathways (p<5e-5 CpGs genes)	Number of genes	P-value	FDR p-value
ABC transporters	3	1.22E-04	2.76E-02
Pathways (p<0.001 CpGs genes)	**Number of genes**	**P-value**	**FDR p-value**
Phospholipase D signaling pathway	21	2.44E-05	6.14E-03
Axon guidance	23	4.42E-05	6.14E-03
EGFR tyrosine kinase inhibitor resistance	14	5.65E-05	6.14E-03
Morphine addiction	14	2.71E-04	2.17E-02
Dopaminergic synapse	17	5.05E-04	2.17E-02
Ras signaling pathway	25	5.16E-04	2.17E-02
AMPK signaling pathway	16	5.42E-04	2.17E-02
Inflammatory mediator regulation of TRP channels	14	6.57E-04	2.17E-02
Choline metabolism in cancer	14	6.57E-04	2.17E-02
GABAergic synapse	13	6.66E-04	2.17E-02
MAPK signaling pathway	29	8.63E-04	2.51E-02

Glutamatergic synapse	15	9.22E-04	2.51E-02
Autophagy	16	1.11E-03	2.58E-02
Thyroid hormone signaling pathway	15	1.11E-03	2.58E-02
Relaxin signaling pathway	16	1.31E-03	2.83E-02
Longevity regulating pathway	10	1.39E-03	2.83E-02
ErbB signaling pathway	12	1.59E-03	3.06E-02
Endocrine resistance	13	1.85E-03	3.34E-02
Proteoglycans in cancer	21	2.09E-03	3.59E-02
Endocytosis	24	2.35E-03	3.83E-02
Fc epsilon RI signaling pathway	10	2.83E-03	4.13E-02
Serotonergic synapse	14	2.85E-03	4.13E-02
Endocrine and other factor-regulated calcium reabsorption	8	2.91E-03	4.13E-02
Sphingolipid signaling pathway	14	3.62E-03	4.91E-02
Cell adhesion molecules (CAMs)	16	3.76E-03	4.91E-02

3.3. Overlap Between PTG and PTSD

To test whether CpGs associated with PTG were also associated with PTSD, the results of the PTG epigenome-wide analysis were examined to check if these CpGs were also associated with changes in PTSD symptoms at the two time-points. At the epigenome-wide threshold, none of the CpGs associated with PTG overlapped with PTSD. Using a less stringent threshold of suggestive significance at $p < 5 \times 10^{-5}$, only the *PDE2A* gene was associated with both PTG and PTSD as shown in Figure 1. A total of 11 CpGs across nine genes were associated with changes in PTG at $p < 5 \times 10^{-5}$ and PTSD at a nominal $p < 0.05$. These included *NRG1*, *TRAK2*, *ABCA13*, *ADRA1A*, *SLC9A10*, *C10orf4*, *SNPH*, *RND1*, *FAM89B*, *RCBTB2* and *C12orf24*.

4. Discussion

This study represents the first longitudinal epigenome-wide study of PTG, exploring associations between changes in PTG scores and DNAm following trauma exposure in first year paramedicine students. Our findings provide novel insights into the epigenetic underpinnings of PTG and establish a foundation for understanding the biological mechanisms that distinguish adaptive post-trauma responses.

The EWAS of PTG identified two CpG sites (cg09559117 and cg05351447) significantly associated with changes in PTG scores after stringent multiple testing corrections at the epigenome-wide level ($p < 5 \times 10^{-8}$). Neither of the implicated genes has been previously associated with PTG, representing entirely novel findings in this field. The cg09559117 site lies within the *PCDHA1* gene body and close to the promoter of the *PCDHA2* gene. *PCDHA1* and *PCDHA2* are members of the protocadherin alpha gene cluster on chromosome five. The protocaderin proteins are calcium-dependent cell-adhesion proteins that are involved in the establishment and maintenance of specific neuronal connections in the brain (Wu & Maniatis, 1999). Interestingly, the PCDH-alpha gene cluster lies downstream and in proximity (< 6.5Mb) to the *NR3C1* locus, a highly conserved human gene locus shown to be enriched in epigenetic changes following exposure to early life stress (Suderman et al., 2012). There are also other reports of the PCDH genes in psychiatric disorders. For example, genetic deletions in the *PCDHA1* have been linked to bipolar and schizophrenia (Lachman et al., 2008). Previous research has found that expression of the *PCDHA2* gene is significantly different in individuals with schizophrenia compared to healthy controls (Shao et al., 2019). In rat models, altered expression of *PCDHA2*

was identified in the brains one month after traumatic brain injury (Paban et al., 2016). The cg05351447 site lies within the *PDZD8* gene body near the 3'UTR of the gene. *PDZD8* has been linked with PTSD in previous genomic research. For example, an allele of the *SLC18A2* gene was significantly associated with decreased expression of *PDZD8* in the dorsolateral prefrontal cortex of post-mortem brains of people with PTSD (Bharadwaj et al., 2018). The identification of *PDZD8* in our PTG analysis suggests this gene may play a broader role in trauma-related outcomes beyond pathological responses.

Pathway analysis revealed that PTG-associated genes were significantly enriched in the Adenosine Triphosphate Binding Cassette (ABC) transporters pathway at the suggestive significance level. This pathway includes genes such as *ABCA13*, *ABCC5*, and *ABCA12*, and has been linked to mitochondrial dysfunction—a proposed therapeutic target for PTSD (Mellon et al., 2018). ABC transporters, particularly ABCB1/P-glycoproteins expressed on brain microglia, may play emerging roles in psychiatric disorders including Alzheimer's disease (Wei et al., 2021). At a less stringent threshold, additional pathways were identified including phospholipase D signaling, axon guidance, and dopaminergic synapse pathways, suggesting complex neurobiological mechanisms underlying PTG.

The candidate gene analysis revealed significant associations between PTG and five genes previously linked to PTSD: *ANK3*, *DICER1*, *SKA2*, *IL12B*, and *TPH1*. This finding was significantly greater than expected by chance (enrichment p-value = 0.003), suggesting shared biological pathways between PTG and PTSD despite their distinct psychological manifestations. *ANK3* and *DICER1* are protein-coding genes that are associated with intellectual developmental disorder and global developmental delay, respectively (Iqbal et al., 2013; Klein et al., 2014). Higher cognitive ability is associated with decreased PTSD symptom severity following trauma (Carlson et al., 2016; Koenen et al., 2007), and higher cognitive flexibility is linked with greater degrees of PTG (Hijazi et al., 2015). As the *ANK3* and *DICER1* genes are associated with cognitive capacity and cognitive capacity influences posttraumatic outcomes, the altered DNAm at these loci associated with changes in PTG represents an interesting avenue for further research. Ankyrin 3 gene (*ANK3*) produces the ankyrin G protein that plays an integral role in regulating neuronal activity. It has generally been associated with various processes including reactivity to stress, impulse control, and memory (Logue et al., 2013) and bipolar disorder (Ferreira et al., 2008). *DICER1* is an enzyme that generates mature microRNAs (miRNAs), which regulate gene expression post-transcriptionally in brain and other tissues; it is also involved in synaptic maturation and plasticity. Lower blood *DICER1* expression was reported to be significantly associated with increased amygdala activation to fearful stimuli which is a neural correlate for PTSD (Wingo et al., 2015). *TPH1* and *SKA2* genes are associated with mental illnesses, including PTSD, personality disorders, anxiety, and depression (Inoue et al., 2010; Neves et al., 2021; Nolan et al., 2000). Mental illnesses are common sequelae following trauma with symptom severity typically reducing with treatment and time. The association between differential DNAm within these genes and PTG could represent a pathway by which downstream effects develop.

When assessing the relationship between PTG and PTSD, there was little overlap in the CpGs associated with both PTG and PTSD. At the epigenome-wide level, no CpGs were associated with both PTG and PTSD after multiple testing corrections. Using a nominal p-value revealed only one CpG site shared between the two posttrauma outcomes. The CpG site cg03929569 is not linked to any gene but exists on an island on chromosome 13. Previous research with monozygotic twins discordant for cerebral palsy found significant differences in DNAm at cg03929569 (Mohandas et al., 2018).

This study has notable strengths. As the first EWAS of PTG, it provides an unbiased, genome-wide perspective that overcomes the limitations of candidate gene approaches. The longitudinal design, assessing DNAm both before and after trauma exposure, better

establishes temporal relationships and accounts for the dynamic nature of epigenetic modifications. This approach provides stronger evidence for causation than cross-sectional studies. One limitation of the study is the small sample size of 39 participants, nevertheless the longitudinal study design employed here is a powerful approach and provides many benefits including assessment of same individuals across two times. While significant genes associated with PTG were identified even after stringent corrections for multiple testing, there were no similar studies in PTG to replicate these results.

These findings have important implications for understanding the biological basis of resilience and adaptive responses to trauma. The identification of specific genes and pathways associated with PTG provides potential targets for interventions aimed at promoting post-traumatic growth rather than merely treating pathology. The distinct biological signatures of PTG versus PTSD suggest that promoting resilience may require different approaches than treating trauma-related disorders. Future research should focus on replicating these findings in larger, more diverse cohorts and investigating the functional roles of the identified genes in neuroplasticity and adaptive responses. Longitudinal studies tracking individuals over extended periods could provide insights into how epigenetic changes associated with PTG evolve over time and whether they predict long-term outcomes.

In summary, the results from this first EWAS of PTG have provided novel insights into the biology of PTG, implicating the *PCDHA1, PCDHA2* and *PDZD8* genes in the aetiology of PTG. The genes and pathways identified in this study can be used in further investigation to provide insight into the etiology of PTG and how it relates to the biology underlying PTSD. Future prospective research within larger cohorts will provide more power to identify additional genes associated with PTG. Ultimately, these findings may inform the development of targeted interventions to enhance post-traumatic growth and resilience in trauma-exposed populations.

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