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Identical Genes, Unique Environments: A Qualitative Exploration of Persistent Monozygotic-Twin Discordance in Literacy and Numeracy

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This study aimed to explore unique environmental factors impacting differential academic trajectories among Australian school students. Monozygotic (MZ) twin pairs who were consistently discordant in results of nationwide standardized tests of reading, numeracy or writing between Grades 3 and 9 were identified. MZ twins control for genes, gender, age, and aspects of the home and school environment shared by twins. Thus, any difference between MZ twins in academic outcomes can be attributed to the unique environment experienced by each twin. From 551 MZ twin pairs with three or four sets of test results, we identified 55 pairs who were substantially and consistently discordant in reading, numeracy or writing between Grades 3 and 9. Parents were contacted for interview, resulting in 40 semi-structured interviews. Qualitative data analysis revealed three major themes, interpreted by parents as possible contributors to persistent academic discordance: biological mechanisms, school-based factors, and personal factors. We discuss implications for educational practice, policy, and research.

Keywords: discordant monozygotic twin pairs, literacy, numeracy, writing, non-shared environment, Naplan research, qualitative interview method

INTRODUCTION

Twin studies of literacy and numeracy development among school students have shown that genes play a significant role in the emergence of individual differences, with estimates of heritability ranging from around 40% to around 75% (Haworth et al., 2007; Oliver et al., 2007; Asbury and Plomin, 2014; Grasby et al., 2016; Little et al., 2017). The same studies have also shown that factors affecting individual twins differently within pairs (usually termed "unique environment") account for most of the variance not explained by genes. Factors that make twins within a family similar to each other and which can differentiate between families (usually termed "shared environment"), such as parental educational values or socioeconomic status, play a more modest role.

In this article we explore this academically-influential unique environment over the middle school years (Grades 3 through 9). We do so by focusing on monozygotic (MZ) twins who differ substantially and consistently in literacy and/or numeracy. Identical twins share the same genetic code (that is, have the same "nature") and typically grow up within the same family (that is,

1

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potentially share family-based aspects of "nurture"), so discordant pairs should provide valuable information about how the unique environment (the unique part of nurture) affects growth in literacy and numeracy.

Background and Context

Behavior-genetic research that has attempted to identify the factors that contribute to the unique environment has met with limited success (see Turkheimer and Waldron, 2000; Plomin and Daniels, 2011; Tikhodeyev and Shcherbakova, 2019). Even in studies where potential unique environment factors have been measured, they typically explain only a small portion of variance (Turkheimer and Waldron, 2000; Plomin et al., 2016). This conundrum has been termed the "missing environment" problem (Asbury et al., 2016), analogous to the missing heritability problem (Manolio et al., 2009).

There are relatively few studies of educationally relevant phenotypes using discordant MZ twins, as noted by Vitaro et al. (2009), and none as far as we can determine exploiting both multi-trait and multi-occasion literacy and numeracy data of the kind we have available. Along with the longitudinal approach that we employ, a novel feature of the current study is an exploration of biomedical conditions that might impact upon academic achievement. The extent to which different biomedical conditions affect school performance has been little explored (Taras and Potts-Datema, 2005; Compas et al., 2017), and the discordant MZ twin design used here is a particularly powerful way of addressing this issue. Biomedical conditions that could contribute to persistent twin discordance in academic domains might include birth complications, injuries, sensory difficulties, or illnesses affecting only one twin.

The only existing qualitative study investigating MZ twin discordance in academic achievement is a single time-point study by Asbury et al. (2016). This study used discordant MZ twins to identify possible unique environment factors affecting results in English, Mathematics, and Science in the General Certificate of Secondary Education (GCSE) examinations that most United Kingdom students sit at age 16. They interviewed members of 65 families in which twins were discordant by two grades (ranging from A* to E) in any of these tests. Family members, including the twins themselves, reported both behavioral and environmental differences as explanations of the discordancy. Behavioral differences were exemplified by personal factors such as commitment to study, interest in the subject, and perfectionism, for most of which respondents did not offer further environmental explanations. Environmental factors were partly school-based, such as perceived teacher quality and assignment to different "ability" groups in school. It is of interest to note that the majority of pairs, 49 out of 65, were discordant on only one of the tests. The most common overlap, 8 pairs, was between Mathematics and Science. Only 3 pairs were discordant on all three tests. With the present investigation, we have the opportunity to explore whether this pattern of relatively little overlap repeats itself with Reading, Writing and Numeracy, the three domains selected for this study.

A small number of studies have attempted to directly measure MZ twin discordance in a range of factors and relate these to discordance in academic achievement. Asbury et al. (2006) used a quantitative discordant MZ twin design and found discordance in birthweight and parental communication was related to discordance in academic achievement at age 7, accounting for 1-2% of the variance. The relationship between discordant parental communication and achievement was stronger when the extreme 10% of twins were examined, accounting for 10–12% of variance. Notably, the unique environmental variance is typically in the order of 25% in the sample from which this study was drawn (Plomin et al., 2012). Likewise, using twins from the same sample, Asbury et al. (2008) explored MZ twins' differing perceptions of the classroom environment and their relationship to English, Maths, and Science achievement. They found no relationship between discordant perceptions and English achievement, but some small relationships between discordant peer problems and Maths achievement (effect size 8%), and between discordant positivity about school and Maths and Science achievement (effect size 15 and 8%, respectively).

Walker and Plomin (2006) likewise investigated 9-year-old twins' perceptions of the classroom environment. Heritability estimates demonstrated the unique environment component to be the most substantial portion of variance in their Classroom Environment Questionnaire, accounting for between 58 and 78% of the variance. However in phenotypic analyses, none of the six questionnaire scales were strongly related to academic achievement, leading the authors to note that their findings "underscore the individual nature of experience, and point to the necessity of research that more closely examines non-shared environment in the classroom" (p. 556).

These results show that the observations by Tikhodeyev and Shcherbakova (2019) of the limited success of quantitative explorations of unique environmental influence remain relevant to educational research. The current study was designed to supplement existing quantitative methods with qualitative ones in a mixed-methods design akin to the model proposed by Power et al. (2018). Specifically, we used quantitative data from a longitudinal twin study of educational outcomes to identify discordant MZ twins, followed by interviews with the twins' parents. The interviews were designed to identify a range of unique environment factors that potentially affect school achievement across three key academic domains, Reading, Writing and Numeracy. Investigating only MZ twins allowed us to control potential confound variables such as genes, gender, age, cohort effects, school effects, and the macro features of family life such as SES and parental attitudes to education.

Mixed-methods investigations such as the current study have the potential to generate and strengthen hypotheses about the unique environment factors that contribute to students' academic achievement over time. The unique environment factors identified in a study of this type can then be subject to further investigation, including quantitative, experimental, and other methods. Our research question therefore was: How do biomedical, personal and school factors contribute to persistent MZ twin discordance in academic achievement?

METHOD AND RESEARCH DESIGN

In this study we explore MZ twins discordant in achievement on at least one of three domains: Reading, Numeracy and Writing. These three domains are assessed in the National Assessment Program: Literacy and Numeracy (NAPLAN). The NAPLAN is a suite of tests administered to Australian school students in Grades 3, 5, 7, and 9 in May each year since 2008. The latest estimate indicates that 96% of students across the country take the tests (Australian Curriculum Assessment and Reporting Authority, 2016). The five tests are Reading, Spelling, Grammar and Punctuation, Writing, and Numeracy. For this study Reading, Writing and Numeracy were selected because they are considered foundational academic skills.

Methodological Issues

Most MZ discordant designs conceptualize discordancy by the presence or absence of a diagnosis (e.g., bipolar disorder or schizophrenia; see Petronis et al., 2003; Kuratomi et al., 2008). However, due to the continuous nature of NAPLAN data we elected to define discordancy as greater than 1 SD on one occasion, and greater than 0.5 SD on all other occasions, with the difference between twins in the same direction at each measurement.

When are differences in a quantitative trait between twins in a pair large enough to count as discordant? This is a practical question that cannot be avoided by researchers using a qualitative approach who do not wish to interview all pairs no matter how small the discrepancy. There is no firm answer to the question, in the same way as there is no firm answer to other cut-off questions such as when a score on a reading test is low enough to count as dyslexia (Spencer et al., 2014). Some leverage might be afforded by the reliability of the trait's test, with highly reliable tests presumably requiring a more modest discrepancy than low-reliability ones to generate confidence that the difference is not simply measurement error. But clearly there is a trade-off: Set the discordancy bar high and eliminate pairs whose discordancy could easily disappear on another test occasion but simultaneously limit the number of eligible pairs; set it low and help recruit a larger sample but simultaneously fail to identify any predictive unique environment factors.

The Australian Curriculum Assessement and Reporting Authority (2013) publishes Cronbach alpha values for the NAPLAN tests, with Reading reported to be in the range of 0.85–0.91, Writing in the range of 0.93–0.96, and Numeracy in the range of 0.86–0.94. In light of these relatively high values, we elected to initially define discordancy as equal to or greater than one standard deviation, with that value based on our twin sample statistics for a particular test (Reading, Writing, or Numeracy) on a particular occasion (e.g., Grade 5 Reading).

The number of test occasions for the twins can vary between one and four, depending on when the pair was recruited into the study. This project was designed to take advantage of the longitudinal nature of the dataset thus we restricted our attention to pairs with three or four measurement occasions, a strategy that goes some way to ensuring that the processes that we do identify have a degree of stability. The next question we addressed therefore was whether a pair should be equally discrepant on all available measurement occasions. Again, there is a tradeoff between insisting on high stability (e.g., discordant by 1 SD at all measurements) and relaxing this criterion. High stability presumably ensures that there is a profound environmental factor in operation but risks missing shorter-lived influences that may nevertheless be important, just as does the decision to omit pairs with only one or two NAPLAN grade scores. Of course, employing a lower level of discordancy across the three or four grades will necessarily catch highly stable pairs but simultaneously increase the sample size. Ultimately the properties of our data largely dictated our strategy: high stability with high discordancy at each occasion just did not occur, consequently we adjusted the criterion to at least 0.5 SD discordant for all other occasions.

Twin pairs could be discordant for Reading, for Writing, for Numeracy, or for any combination. We did not commit to a high degree of generality as a condition for inclusion in the study, a decision independently motivated by the observation of only modest overlap in discordancies in Asbury et al. (2016). That decision was supported by finding only two of fifty-five pairs discordant for all three domains, and six pairs discordant for two domains. These patterns jointly confirm a degree of specificity in environmentally-sourced variance in academic achievement, in line with the rubric that genes are (fairly) general but environments are specific (Kovas and Plomin, 2007).

To provide a sense of the scale of our selected discordancies, note that the expected national gain in reading achievement from Grades 3 to 5 is approximately 75 points (Australian Curriculum Assessement and Reporting Authority, 2016). Thus, 1 SD difference in achievement in Reading at Grade 3 in our data (87.67, see **Table 1**), exceeds the expected gain for this cohort between Grades 3 and 5. A difference of 0.5 SD in MZ twin pairs indicates that one twin is performing \sim 1 year behind their cotwin. These patterns are consistent for the three domains and four testing occasions reported here insofar as 1 SD is greater than the expected gain over 2 years of school.

TABLE 1 Numbers of participants, means, and standard deviations by grade level and domain

Grade	Reading		Numeracy		Writing	
	n	M(SD)	N	M(SD)	n	M(SD)
3	3,404	451.14 (87.67)	3402	424.63 (74.24)	3404	433.23 (62.56)
5	3,530	527.14 (78.48)	3527	512.43 (70.89)	3532	499.34 (64.82)
7	3,356	576.91 (67.92)	3351	575.48 (71.46)	3356	546.26 (70.79)
9	2,855	616.42 (66.74)	2826	621.43 (71.09)	2849	590.09 (82.25)

Means and SDs were calculated using the entire sample of twins before selecting MZ pairs with three or four test occasions.

Qualitative Methodology

The qualitative approach taken was Theoretical Thematic Analysis (Braun and Clarke, 2013) in that analysis of the data was informed by theoretical constructs in the field of behavior genetics, and the process of data analysis aimed to identify common themes in an attempt to answer the research questions. The researchers were motivated by a gap in theoretical knowledge in behavior genetics: what factors constitute the unique environment portion of variance in behavior-genetic studies of educational attainment. In this sense, the research paradigm is post-positivist: we accept that currently-unknown environmental factors affect twins' development uniquely, that is, they serve to make MZ twins different from each other; we accept also that such factors are identifiable and (ideally) quantifiable (Kivunja, 2017). These factors, if identified in research such as this, can then be measured and applied at the level of variables in quantitative analysis.

Participants

Participants were obtained from the cohort sequential Longitudinal Twin Study of the NAPLAN. We hold results for the five NAPLAN domains for any of the grades (3 through 9) for a total of 2,528 sets of twins (47% MZ). Of the entire sample of MZ twins, 52% are female. We recruited any twins with NAPLAN assessment scores at any grade between 2008 and 2017.

Monozygotic twin pairs who were consistently discordant in at least one academic domain across time were selected by applying the following criteria: pairs had completed three or more NAPLAN test grades, were discordant by >1 SD on at least one occasion, and remained discordant in the same direction by >0.5 SD on all additional test occasions. Means and standard deviations were calculated for each domain using the whole sample of twins (see **Table 1**). There were 551 MZ pairs (53% female) with three or four measurement occasions. Of the 551 pairs, 208 (38%) were discordant in Reading by at least 1 SD on at least one occasion, with 183 (33%) discordant in Numeracy and 354 (64%) discordant in Writing applying the same criteria.

We treated a value of 1 *SD* discordant as "entry-level" for a pair to be candidates for discordancy. After applying the criteria of discordancy of at least 0.5 *SD* on all remaining test occasions, we were left with 60 pairs across the three test domains. After removing pairs with uncertain zygosity information (5 pairs), 55 pairs (27% female) were candidates for follow-up interviews. At the time of interview with a parent, twins ranged in age from 13 years, 2 months to 20 years, 4 months. Zygosity was determined by DNA testing in 58% of cases, and parents' confirmation of twins' physical similarity provided in an earlier questionnaire (see Grasby et al., 2016 for details). In **Table 2** we report frequencies of discordant pairs for each domain.

Only 8 pairs of 55 (15%) were discordant in more than one domain. These were specifically: two pairs discordant on all three domains; two pairs discordant on Reading and Numeracy; three pairs discordant on Reading and Writing; and one pair discordant on Writing and Numeracy. The corresponding percentage of discordancy overlap on the tests in Asbury et al. (2016) was 25%. These data in turn suggest

TABLE 2 | Number of pairs identified as discordant in each domain.

	Numeracy	Reading	Writing
Identified	19	23	23
Interviewed	16	17	13

Values in each column represent the total number of pairs identified as discordant. Pairs could be discordant in more than one domain.

that unique environmental factors are only weakly shared across academic domains.

Measures

The NAPLAN tests are aligned with the Australian National curriculum and are designed to assess student abilities at each grade level in the key areas of literacy and numeracy (Australian Curriculum Assessement and Reporting Authority, 2017).

Questionnaire data is collected from the twins' parents and covers topics including parent education, parent attitudes to literacy and mathematics, twins' health, diet, sleep habits, media use, homework habits, scores on measures of attention and hyperactivity, and aspects of twins' educational histories such as preschool attendance and classroom separation in primary (Grades 3 and 5) and high school (Grades 7 and 9). Quantitative results from this project are reported in Grasby et al. (2015, 2016, 2017), Grasby and Coventry (2016), and Gould et al. (2018).

For this study, a semi-structured interview protocol was developed (see **Supplementary Material**), informed by a review of the literature investigating possible unique environmental factors that influence academic achievement (Walker and Plomin, 2006; Asbury et al., 2008, 2017; Greven et al., 2009; Asbury and Plomin, 2014; Kovas et al., 2015, 2016).

Procedure

Once twin pairs who met the discordancy criteria had been identified, parents were emailed an invitation to participate in a telephone interview. Of the 55 identified discordant pairs, we contacted the 44 families that we could locate, and of those 40 agreed to a telephone interview of one or both of the parents. We obtained written informed consent from parents of participating twins upon enrolment into the study, including consent to be contacted for an interview, and further verbal consent from participating parents at the beginning of each interview.

Interviews lasted between 30 and 60 min and the semistructured design allowed researchers to follow themes of interest as the interviews progressed. Three pilot interviews were conducted with parents of twins who fell just outside the inclusion criteria, allowing us to test and refine the topics included and rehearse the procedure for the interviews. For consistency, the same researcher conducted all but one of the interviews, and 95% of the interviews had between one and three additional researchers present. The telephone interview format allowed all researchers to take notes unobtrusively during the interviews, and immediately post-interview detailed field notes were composed and emerging themes discussed. Interview notes were also triangulated with data provided by parents in earlier

questionnaires (Creswell and Miller, 2000) in order to further validate the themes emerging from the interviews.

All interviews were recorded with the consent of the interviewee, in 95% of cases the mother of the twins. Following the process outlined in Halcomb and Davidson (2006), audio recordings of interviews were subsequently reviewed by one of the researchers alongside the field notes to ensure the notes represented the content of the interviews as accurately as possible. The process of using audio files rather than text transcriptions of interview data was selected in order to retain the voices of participants in the analysis procedure and avoid misinterpretation through the loss of contextual cues (Crichton and Childs, 2005; Tessier, 2012; Neal et al., 2014). The review of the audio data involved composing a separate set of detailed notes and transcribing direct quotations relevant to the emerging themes. Field notes, detailed notes and quotations were then coded by three of the researchers thereby generating a set of explanatory factors.

The content analysis was an iterative process in which themes would arise in interviews as they progressed, and the process of reviewing the audio data and notes allowed a more detailed analysis and validation of these themes. Secondary content analysis was undertaken on a random sample of 20% of the interviews by a trained researcher who was not involved in the study design or data collection. In this process, a review of audio data and field notes tested and validated the themes that were developed in the interview and review process. Interrater reliability checks were performed on this subset with 88% overlap between the two researchers identifying explanatory factors for twins' discordance (i.e., in 8 interviews, of the 64 factors identified by one researcher, the second identified 56 of the same factors).

Finally, we retrospectively compared the data collection and thematic development procedures followed in this study to the rules for attaining data saturation in qualitative interview studies outlined by Braun and Clarke (2013). This indicated that the number of interviews met or exceeded the number required for data saturation—the point after which additional interviews fail to generate new themes. Specifically the final eight interviews generated no new themes or sub-themes.

RESULTS

In most cases, parents agreed that twins had performed differently in the identified domain and offered explanations as to why this was the case. In three cases, parents reported that the discordance in NAPLAN results did not reflect the achievement of the twins. We also mention here that 73% of twins identified as discordant in this study were male pairs. We have no ready explanation for this imbalance.

Themes emerging from the interview data were categorized into three broad areas, with two of these containing sub-factors (see **Table 3**). Biomedical explanations of MZ twin discordance in academic achievement emerged as a key theme from the parent interviews. Additionally, personal and school factors emerged as possible contributors and reflect the findings of Asbury et al. (2016). We turn first to the biomedical explanations theme.

TABLE 3 | Explanatory Factors Identified by Domain.

Explanatory	Discordant domain (number of families interviewed)					
factors	Numeracy (n = 16)	Reading (<i>n</i> = 17)	Writing (<i>n</i> = 13)			
BIOMEDICAL FAC	TORS					
One twin affected	7	6	2			
Both twins affected to a different degree	3	4	5			
PERSONAL FACTO	ORS					
Effort and motivation	7	4	3			
Interest and enjoyment	4	10	5			
Personality	8	3	2			
SCHOOL FACTORS	S					
Teachers	8	6	4			
Class Allocation	6	3	_			
Peers	5	5	2			

Values in each column represent the total number of families that mentioned this factor as an explanation for discordance. Families typically mentioned multiple explanatory factors and/or sub-factors.

Biomedical Factors

Biomedical factors emerged as an influential theme in 58% of cases. Instances where one twin was affected by a medical condition and the other not were the clearest to interpret as driving forces in persistent discordance (n = 13). In other cases, both twins were affected by biomedical conditions but not to the same degree (n = 10).

Biomedical factors were consistently perceived by parents as causing some kind of deficit in one twin, such that they always lagged behind their co-twin due to the medical condition and associated complications. However, the occurrence of pairs in which both suffered the same condition but displayed differing degrees of severity reinforces the complexity of the effects of such conditions, even in genetically identical pairs. There are exceptions to the rule as well: in some cases, the twin who had more difficulties at birth, such as lower birth weight or breathing difficulties, went on to be the high performer in at least one academic domain.

In just 2 of the 23 cases parents described the biomedical conditions as the sole driver of twin discordance (Crohn's disease and cerebral palsy in just one twin). In the remaining 21 cases all of the additional sub-factors relating to school and personal factors were mentioned at least once, with no tendency for biomedical factors to cluster with particular school or personal factors. Interestingly, in only a minority of cases (6/17 Numeracy, 7/17 Reading, 6/13 Writing discordant pairs), no biomedical factors were proposed by parents as possible contributors. We discuss the implications of this pattern below.

Conditions Affecting One Twin

Parents in 13 interviews identified a number of conditions affecting the poorer performing twin including cerebral palsy, epilepsy, Crohn's disease, tinnitus, sleep apnoea, motor delay,

stroke suffered by one twin at birth and often a combination of two or more of these diagnoses. In all of these cases, the parents were unequivocal in their explanation of the condition suffered by the affected twin as the main contributing factor in the persistent underperformance in school achievement relative to the co-twin. Parents also spoke at length about the difficulties endured by one twin, but not the other, and the additional support they provided to the affected twin during their schooling. In describing the differences in academic achievement during the middle years of school between one such set of twins, one parent was definitive:

I think it [twin's poorer performance] was more related to his health as opposed to any of the other...factors.

Notably, in the two cases where twins were discordant for all three domains, the poorer performing twin had been diagnosed with a severe medical complaint: a brain tumor in one pair and Twin-to-twin Transfusion Syndrome leading to cerebral palsy in the other. Neither of these families responded to our request for interview; they had described the situations in their written responses to our initial family questionnaire. However, we hypothesized that, unlike the interviewed cases, the complications suffered by each twin in these two cases were so severe as to affect all domains assessed by NAPLAN. The most striking conundrum from the interviewed cases is why such conditions do not more often affect all academic domains—in each of the interviewed cases, twins were discordant on only one domain.

Related to the effects of medical conditions affecting one twin was the emergence of parents' descriptions of the adverse side-effects of drugs prescribed to manage these conditions. In particular, parents of a twin with epilepsy and another who developed Crohn's disease both felt that the side effects of the drugs prescribed to manage these conditions were responsible for the delay in the normal development of academic skills in their children. One parent noted the comments of the twin when he switched to a new drug to manage his condition:

For the first time I feel really clear [in thinking].

Similarly, recent research has identified the possible adverse effects of early childhood general anesthetic on academic performance later in childhood, particularly in numeracy (Schneuer et al., 2018). Interestingly, in the three cases where one twin was given a general anesthetic before age 4, earlier, more frequently or exclusive of the other twin, the former twin underperformed in numeracy compared to the latter twin. The parents did not put much emphasis on this occurrence when describing the differences between the twins' performance and yet it is notable that these were all cases where no contributory factors were able to be identified by the parent.

Conditions Affecting Both Twins Differentially

Biomedical conditions suffered by both twins but that affected each twin differently were another theme arising in 10 interviews. Examples of such conditions include Asperger's syndrome, generalized gross and fine motor delay, and the development of hearing, speech or vision problems in early childhood. One mother whose twins both developed Asperger's and motor delay problems described the difference thus:

[Twin 1] has had, you know, the same problems as [Twin 2] all along but just to a lesser degree.

While the effects of this difference throughout the twins' school years were described in great detail, the underlying reason for this difference in degree was unable to be identified.

A similar situation holds for the twins whose parents identified discordant problems with vision, speech or hearing (n =6): the discordance in degree of problem was identified, the interventions to correct the problem were described, but the reason(s) for the discordance was not clear or identifiable. Such problems included one twin requiring glasses, both twins attending speech therapy but one displaying more delay than the other, and one twin suffering from multiple instances of ear infections in the early childhood years. While these problems were generally minor and were always treated and corrected where possible, their incidence has potential long term impacts. In particular, it emerged that interventions designed to solve such problems can interact with the development of a twin's personality and self-perception in ways that are not immediately apparent even to the closest family members. In one interview, the mother described how both twins required speech therapy for language delay, but one twin (subsequently the poorer performer in reading and writing) stubbornly refused to engage with the sessions despite the ongoing efforts of the parents and their recognition that the intervention was important for the development of language and reading skills.

A further theme that emerged from parents' descriptions of the complications arising from fine motor delays was the incidence of extremely poor handwriting. In four cases the poorer performing twin, and in two cases both twins (all male pairs), were described as having "shockingly poor" handwriting. One parent suspected that the twin's poorer performance in mathematics relative to his co-twin was partly down to his "illegible" writing, that he made mistakes in his working because he couldn't read over what he had written. Another parent expressed a concern that teachers had tended to "pigeonhole" the twin because of his physical clumsiness and poor handwriting arising from epilepsy, rather than assessing his ability. Handwriting remains a fundamental skill in school settings, particularly in exam situations, and these cases highlight possibly overlooked factors that can play a role in achievement over time.

Twin-to-twin Transfusion Syndrome (TTTS) was reported in four cases and suspected in another 2 cases (15%). In four cases the recipient twin went on to persistently outperform the cotwin in at least one NAPLAN domain. However, in two of the confirmed TTTS cases the recipient twin was not the higher performer. These findings are relatively unsurprising: TTTS is diagnosed in 10 to 15% of monochorionic twin pregnancies (Umstad et al., 2016) and both donor and recipient twins are at

risk of further neurological complications such as cerebral palsy (van Klink et al., 2013).

Higher Achievement of Affected Twin

In another four cases the twin suffering from a biomedical condition developed motivation and persistence seemingly because of the difficulties they endured. In these cases parents described how the affected twin was more conscientious, more motivated, more persistent, and given more intensive help and support by the parents. In one case, the twin who developed a chronic illness also performed more highly than her cotwin in Reading. She was characterized by her mother as more motivated and "determined to do things," as well as "a very big bookworm-95% of the time she has her nose in a book." By contrast her twin, who did not develop the same illness, was better at sport, more "lively and energized" and would rather watch movies than read. Another parent expressed the sense of relief that the twin who suffered from cerebral palsy and consistently underperformed in numeracy relative to his co-twin had persisted with his writing to the extent that he had begun to receive higher grades in English in high school:

It is encouraging for him because as you can imagine with twins where one consistently outperforms the other it... does raise all sorts of dilemmas in the family and it is something that [he] now also really holds as one of his personal strengths, that he is good at English and in particular good at writing.

These cases provided a counterpoint to the majority of cases where biomedical complications contributed to underperformance in academic domains.

Personal Factors

Parents explained the discordant performance of the twins wholly or partly in terms of personal differences in 33/40 cases (83%). While these behavioral differences and the ways they impacted on their school achievement over time were described in detail, only one of the parents had a possible explanation for how such differences arose. In this case, the mother felt that the father had always treated the twins differently, and that one of them had been his definite favorite:

Right from the word go, [dad] always considered [Twin 2] his twin \dots [he] was allowed to get away with things [but Twin 1 had not].

By contrast, in response to the question of why the twins developed different personal characteristics, all other interviewed parents made comments similar to the following:

I can't really...describe it—I just think they're just different, they've got different characteristics, that's all I can put it down to. I really don't know... they were both raised the same, with the same parents.

Other parents were more specific about having treated the twins as different people:

I don't always expect them to be the same ... I treat them as very different people, and that's how they've grown up.

Whether this differential treatment by the parent(s) was an underlying factor in the development of different personalities, or whether the twins were treated differently *because* they had differing personalities is unclear. Nonetheless, such differences between the twins commonly exist, are particularly salient for parents, and are perceived as a significant factor in school achievement for a large percentage of those interviewed in this study.

The ways these personal differences impacted on school achievement were categorized into three sub-themes, echoing the findings of Asbury et al. (2016):

- Effort and motivation
- Interest and enjoyment
- General personality differences

It is notable that when parents were initially asked in general terms to explain the discordancy between their twins in the identified subject area, for the most part they began by explaining the personality differences between them.

Effort and Motivation

A persistent theme arising in 13 interviews was the description of the higher achieving twin consistently putting in more effort at school or in their homework than the co-twin. Parents used words like "perfectionist," "motivated," "studious," "focused," and "conscientious" when describing the higher achieving twin; conversely the lower achieving twin was described as "lazy," "apathetic," or lacking in effort, completing tasks because they "have to," or giving up if tasks were too difficult rather than persisting. One parent's comment was echoed through a number of interviews:

[Twin 2] works harder ... He's got a perfectionist kind of nature that he'll work hard whereas [Twin 1] is casual—he'll just get the job done. Kind of different personalities.

Comments such as these were always general in nature, applying to school on the whole, rather than specific to the domain in which the twins differed. For example another parent noted:

I think it's just her personality, she just likes to be the best at everything she does, whereas [Twin 1] is ... definitely more laid back.

One parent articulated the development of a positive feedback loop whereby the achievement of one twin motivated him to continue to excel:

[Twin 2] was one of those kids, the better he did, the more he wanted to do better. [Twin 1] was capable but his care factor wasn't as high as his brother's.

When pressed on the causes of such differences between twins, parents were not able to identify why or how these differences

arose, just that they had existed, often from early childhood and prior to commencing school.

Interest and Enjoyment

Twins who displayed greater effort or motivation in general often were also noted to be more interested in the particular subject in which they outperformed their twin. In five cases of Numeracy and/or Reading discordant twins, the parents noted that, not only did the higher performing twin commit more effort at school in general, but they also enjoyed reading more, or were more interested in maths and science subject areas than their co-twin.

Enjoyment of reading specifically was also noted frequently by parents of twins who performed more highly in the Reading or Writing domains, exclusive of comments about effort and motivation. In ten cases (25% of all interviews), difference in performance in either or both of these domains was attributed to the greater interest in reading in the higher performing twin. In comparing the interest levels of the twins, one mother commented that while she did not know why one of the twins developed a love of reading:

He would literally read anything after he became voracious. Another mother was almost puzzled by her son's passion for reading, saying neither she nor her husband were "bookworms," and noting this clear delineation between the twins:

[Twin 2] loves reading, but [Twin 1] not – he doesn't love it ... [Twin 2] reads quite a bit [Twin 1] doesn't really read a lot... [Twin 2] would read a thick novel that would take me 5 years to get through.

Research shows that extensive practice in reading is one of the essential factors in children's development from novice to expert readers (Castles et al., 2018), so this phenomenon is perhaps to be expected insofar as results in reading tests go.

For many twins, interest and enjoyment were described in more general terms, and were tied in with dichotomous descriptions of the twins' personalities. In five of the interviews, contrasts were drawn between the "academic" twin and the "sporty" or "outdoorsy" twin, the twin interested in science and maths and the twin interested in creative pursuits—with the former always the twin who achieved more highly in academic domains. One mother described how this type of difference was apparent even from the very beginning of the twins' kindergarten year:

[Twin 1] just didn't really enjoy being in a classroom, sitting there reading, he didn't enjoy it. Whereas [Twin 2] ... was excited by school whereas [Twin 1] wanted to be outside playing with a ball.

Again a positive feedback loop developed as early as the Kindergarten year where the higher achieving Twin 2 "was reading little books and [Twin 1] was struggling" and developed a level of school anxiety. An extreme example of this kind of differentiation emerged with one set of twins where the twin who underperformed on all but one of the NAPLAN tests from Grades 5 to 9 lost all interest in school and dropped out at Grade 10, while

his co-twin was accepted into a selective school at Grade 7 and went on to achieve good results in his Grade 12 exams.

Other Personality Differences

Three more general personality factors that impact on school engagement and achievement emerged from the interviews. These factors appeared to interact with School factors (described below), but emerge from personalities of the twins, rather than the school environment. The first was a willingness on the part of the higher achieving twin to conform more readily to the expectations of the school. These twins were described as more confident, more "adult oriented," completing homework more readily and having better relationships with teachers: one mother commented:

He seems to be a teacher's pet... the teachers always love him and he ... seems to get all the good teachers. He just got a really good run in Primary school.

By contrast, their co-twin was described as "naughtier," the one who pushed the boundaries more, did not complete homework, or would not put in effort if they could not see the relevance of a task. For example, one mother described her son as lazy, but qualified this saying,

When I say lazy, I don't mean physically lazy, but as a student he's never seen the point, like what's in it for him to study hard at this point?... I think it took a few years of Primary school for that difference [between the twins] to come out.

The second factor was an unwillingness on the part of the lower achieving twin to ask for help when they needed it. In one pair of twins who both achieved very highly in the Numeracy domain, the twin who lagged behind his brother was described thus:

If he finds himself in a situation where he's not understanding he doesn't necessarily ask questions and so on ... therefore he doesn't get help if he needs it.

Thirdly the development of anxiety in the lower performing twin relating to schooling in general, or specific to exam situations, was discussed by parents in seven of the interviews. In all of these cases anxiety appeared to be related to the academic self-concept that developed and was framed in terms of the way the anxious twin perceived themselves in relation to their co-twin. A common theme emerged where the lower performing, more anxious twin would say they were "dumber" than their co-twin, or that the co-twin was the "academic" one. The lower achieving twin in these instances was also often described as lacking interest or effort with one parent suggesting that the twin took this approach to have an excuse for underperforming relative to his higher achieving co-twin:

If he knew he couldn't beat his brother he wouldn't try as hard. It often took a number of years for the lower achieving twin to accept the difference between themselves and their twin and some felt a sense of unfairness because they were not the same as their identical twin.

School Factors

School factors emerged in 27 of the interviews (68%) and could be categorized into three broad and interlinking sub-factors:

- Teachers

Largen et al

- Class allocation
- Peers

It is important to note the reciprocity of school factors with the personal and biomedical factors described above. While many of the reported events appear particularly advantageous to one twin or disadvantageous to the other, much of the time these school factors reinforce already emerging individual characteristics or achievement trajectories. That is, school factors were never described by parents as the only contributor to the discrepancy between the twins in their achievement. Notably school factors relating to teachers and class allocation arose more frequently in interviews where twin pairs were discordant in numeracy (11), rather than reading (4) or writing (4).

Teachers

In 11 of the interviews, parents described factors relating to different teachers as a possible contributor to twins' divergent achievement over time. Some parents perceived the lower achieving twin had experienced "worse" classroom teachers than the higher achieving twin; in other cases the reverse applied: the higher achieving twin had encountered more experienced or motivated teachers. One parent described how one twin had a series of poor teachers in the very early grades of school who were both subsequently demoted, whereas the co-twin's teachers were "fantastic." Another parent commented:

Grade 5 for [Twin 2] was an absolute dreadful teacher \dots so [he] swapped classes in Term 4.

Parents felt that experiences such as these affected the engagement with and enjoyment of school for the twin involved, as well as their learning trajectory.

While the above experiences were framed in terms of the teacher's competence, other parents described how one twin's relationship with their teacher was more difficult, thereby affecting their engagement and learning. One parent felt that the lower achieving twin had experienced a teacher he didn't "gel" with during the first year the twins were separated into different classes. Another parent felt the lower performance of one twin during primary school was "definitely [relating to] the teachers." While this parent did not feel the primary school teachers had been especially good for either twin (both very keen on sport), she felt the lower performing twin had experienced worse teacher relationships:

In Grade 5, I know the teacher spent a lot of time with all the kids who were academic and the kids who were sporty she didn't really ... worry about.

The impact that teachers can have on the developing academic self-concept of children was clearly expressed by another mother who felt that while both twins had experienced good teachers in separate classes in early primary school, the higher achieving twin had a very supportive teacher who had developed her confidence in her own ability. By contrast:

[Twin 1] didn't get that confidence boost that [Twin 2] got... and sort of learn that she was clever or stood out in any way...[Twin 2] has that confidence that [Twin 1] is lacking.

Their mother felt the impact of these formative years had been long lasting, and were compounded when Twin 2 was accepted into an extension class in Grade 7 and Twin 1 was not.

Class Allocation

The streaming of one twin into an accelerated or extension class was a strong theme in six of the interviews. In all of these cases, two other factors were consistently identified as playing a part in the differential achievement of these particular twins: chronic illnesses suffered by one twin and not the other, and the more conscientious twin being assigned to the extension class. In any case, whatever the triggering factors, assignment to advanced (vs. regular) classes seemingly had the effect of "cementing in" students' perceptions of their abilities.

In all but one of these instances, streaming on apparent academic ability was a feature of secondary school, not primary school. In all examples, streaming into higher ability classes was not viewed by parents as the defining factor in the different performance of the twins; rather twins had already begun to demonstrate different levels of achievement and the academic streaming reinforced the higher achievement of one twin. One mother felt that the twin in the high achieving classes consistently had "better" teachers and that the lower streams not only included more disruptive classmates but were also allocated the "less good" teachers. Another parent felt that the streaming of the twins had meant that "they're now permanently set up with different skills" because the twin in the accelerated class had more opportunities to do extension work and move through the curriculum at a faster pace.

Five parents also spoke about the general effects of first separating twins into different classrooms early in primary school. While this could be an issue specific to twins, it is important in this context because such class separations can have impacts on twins' emotional and behavioral adjustment at school, and subsequently on their achievement (Tully et al., 2004). Parents who identified class separation as a potential factor in twins' differential achievement trajectories did not necessarily identify differences in teacher quality as an additional factor. Twins were separated for a variety of reasons: to develop independence, because their behavior was too disruptive when they were together, to avoid competition between them, or because differences in achievement were already apparent. One parent felt that the classroom allocation in the first grade the twins were separated had a long term effect: one twin was placed in a composite class with an older grade, and the other twin was placed in a composite class with a younger grade. The latter twin thereafter felt he was not "as smart" as his twin, despite the class allocation being a product of circumstance.

Peers

In 10 of the interviews, parents identified peer group effects as possible influences on the achievement of the twins. Commonly, parents described the twins gravitating toward friends with similar interests, for example the "academic" friends and the "sporty" friends reinforcing already established preferences. At the extreme end was a case where one twin, who subsequently dropped out of school, was involved with a peer group that engaged in antisocial activities including drug and alcohol consumption and graffiti, whereas the higher achieving cotwin attended a different school and was not involved with the same peers. Another parent described how the higher achieving twin "gets in with all the top nerds" and they compete with each other in academic domains, whereas the co-twin's friends are "more casual and relaxed." In a group of four cases, all female twin pairs, parents described social problems with peer groups and bullying behavior toward one twin as having a detrimental impact on the school achievement of the affected twin.

DISCUSSION

One of the enduring conundrums in psychological research involving twins is why and how identical twins can develop so differently (see Plomin and Daniels, 2011). This study attempted to pinpoint the factors that affect MZ twins to an extreme extent: twins were widely discordant on standardized tests in at least one of three academic domains, and the discordance was consistent over at least three biennial testing occasions. Recall that 0.5SD is equivalent to $\sim\!1$ year's growth as measured by NAPLAN tests, and all sets of twins interviewed in this study were discordant by at least 0.5 SD on three or four occasions. The discordant MZ twin design allowed us to control for genetic influences, gender, age, and shared environment factors.

We have reported three broad areas that emerged as possible unique environment contributors to extreme discordance in NAPLAN achievement: biomedical factors, personal differences and school factors. Most parents (90%) confirmed the discordance identified in the NAPLAN data and described factors relating to one of more of the three identified themes as contributors to their twins' achievement trajectories. Notably, parents did not confine their explanations to domain-specific differences between twins, despite the focus on discordancy in (usually) one domain at the outset of the interviews.

In behavior genetics, unique environmental estimates encompass both environmental influences that serve to make members of a twin pair less similar and error, though it is often difficult to discern between the two. However, this MZ discordant design, which insists on discordancy across three to four measurement occasions, is better able to disentangle true environmental influences from error. Thus, the factors revealed here might be considered as those that have persistent and long-lasting impacts on school achievement.

Aside from biomedical explanations, the factors that commonly emerged were those that have been extensively explored in educational research in relation to their effects on achievement: motivation, conscientiousness, teacher quality, teacher relationships, ability streaming, and peer effects. It is clear from this study that personal differences are associated with achievement and interact reciprocally with school and peer factors. A number of twin studies have identified the dominant role of unique environment factors in explaining variance in self-perceived abilities, motivation, and perceptions of the classroom environment (Walker and Plomin, 2006; Greven et al., 2009; Kovas et al., 2015). It is therefore unsurprising that personal difference explanations should emerge from the interviews. Why and how such differences first arise in MZ twins is much less clear, despite this being a specific goal of this study.

It is important to recognize the complexity of definitively identifying the influences of each of the factors described above and the associated difficulty of reducing them to measurable variables. None of the factors occur in isolation; instead they co-occur and interact in ways that are at times unexpected and unpredictable. Biomedical conditions influence the development of personality, academic self-concept, and peer and teacher relationships, in what could be seen as "biology-environment correlation," analogous to the gene-environment correlation frequently invoked in behavior-genetics. Likewise, personality factors affect peer and teacher relationships. Classroom allocation and "ability" streaming can be both caused by and reinforce already apparent differences in achievement. While parents were not always able to articulate this, the interviews demonstrated that a small difference could develop over time into a clear delineation that is integrated into twins' perceptions of themselves and their abilities.

Implications

In this section, we outline what we see as some of the implications of this research for educational practice, policy and research. We base our suggestions, first, on the central message of the data—that there can be wide variation in educational achievement, up to 2 years' growth with our participants, in the face of constant ability levels, with "ability" here as code for genetically-influenced educational potential. We also base them on specific observations of parent-reported reasons for accelerated or diminished achievement, as we argue below.

Teachers

Some have argued teachers would benefit from being better informed about genetics (Chapman et al., 2018; Crosswaite and Asbury, 2018). Improved genetic knowledge and a parallel resistance to a genetic fatalism matter because, as we have shown, genes are not destiny when it comes to literacy and numeracy during the middle school years. Thus, we encourage teachers to remember the message that, despite known substantial levels of genetic influence on literacy and numeracy, many factors in the student's environment are also influential, including encouragement and direction from teachers. Their resolve to help all students achieve high levels of these vital academic foundations should not be undermined by an appreciation of a role for genes—that resolve should be bolstered by our findings that there are also substantial roles for the environment.

It is also worth noting that, if parent reports are accurate, students can be affected by perceived attitudes of teachers toward them. It is challenging for teachers to feel equally positive about all students in a class, but attention to possible negative consequences of differential treatment of students, whether intentional or not, would be an important part of teachers' mindsets.

Policy

Placement of twins into the same or separate classes was a common theme in the interviews. Schools typically do not have a policy one way or the other on this matter, leaving the decision to parents when the twins are young and, increasingly, to the twins themselves as they progress through school. We endorse this "hands off" policy but emphasize that teachers, administrators and parents need to keep each case under review, particularly in the early years, as the twins themselves may not react in the ways anticipated. It may be comforting to know, however, that available research on academic effects of separation indicates that there are no significant, systemic effects on academic outcomes of separating vs. keeping twins together (White et al., 2018).

Academic streaming emerged as a theme, with the consensus that when it occurred it was a consequence rather than a cause of discordancy. But there also appeared to be a reinforcing effect of streaming, such that twins' academic self-concepts as good or less good at a school subject became more firmly fixed. Thus, there is the potential for students not allocated to an advanced stream to see themselves as less able than they truly are. These results align with a large body of international research demonstrating that while streaming may be academically beneficial for high achieving students, the practice is largely disadvantageous for those students not streamed into the more advanced classes (Johnston and Wildy, 2016).

Research

Some of the unique environmental factors that we identified, such as motivation, academic self-concept, and peer relations, are the subjects of extensive educational research (see summaries such as Blatchford and Baines, 2010; Boekaerts et al., 2010; Marsh and Retali, 2010). Our contribution to these endeavors is just this: These factors are not simply proxies for ability; they can and should be studied in their own rights, knowing that they can play roles in how well students manage academically over and above any genetic potential that may exist. We encourage researchers to continue to probe such factors, doing what they can to control for genetic potential. In the future, that control may include polygenic scores (Lee et al., 2018).

It is important to reiterate the value of qualitative data such as these in terms of generating new hypotheses for future research (Power et al., 2018). MZ twins are a particularly stringent approach to identifying such hypotheses because the results are not confounded by genetic effects. We have described an alignment between the results of this study and a large-scale population study of the effects of anesthesia in early childhood (Schneuer et al., 2018). It would be valuable to interrogate other twin databases to further explore the effects of anesthesia on academic achievement, controlling for genes by using discordant

MZ twins. Further areas of research suggested by the themes arising in the interviews include the possible side effects of drugs prescribed for biomedical conditions such as Crohn's disease and epilepsy that could be detrimental to academic growth, and whether the effects of poor handwriting plays into teacher perceptions of student ability and consequently achievement.

Biomedical factors were mentioned as possible contributors to twin discordance in the majority of cases. Although we cannot be sure that the proposed biomedical factors did in fact play a role in these cases, best practice in educational research might comprise a more complete collection of students' medical histories, including the frequency of apparently minor ailments such as ear infections.

As noted earlier, there were more male pairs identified as persistently discordant. Future research might further explore whether this gender imbalance relates to certain aspects of learning engagement specific to boys in the middle school years.

Limitations

Veracity of qualitative data is a limitation for this study. Indeed, it has been famously argued that verbal reports of one's own mental processes are inherently limited (Nisbett and Wilson, 1977) and it is likely therefore that some of the reasons for twin discordancy remain hidden from parents' (and the twins') conscious access.

In the present study one informant, the twins' mother, reported for twin pairs in all but two of the interviews (one interview with the father, and one with both parents). In an overview of discordant MZ designs, Vitaro et al. (2009) suggest that multiple informants are preferred because single informants will tend to rate MZ twins more similarly. In addition, twins themselves may have different interpretations, and an extension of this study would seek to interview twins, parents and teachers and compare the themes arising from different informants. The fact that the parents already knew the existence and the direction of the discrepancies in the academic performance of their children, also gave their interpretations a purely a posteriori nature, with the limitations this holds. Nevertheless, two of the major themes arising from the interviews reflect earlier work (Asbury et al., 2016), so we remain confident that these findings are informative despite our reliance on one parent.

This study was concerned with why twins differed from each other, rather than why twins achieved particular scores, consequently the themes that emerged from the data might not relate specifically to higher or lower than average achievement in general. There may be different dynamics at play in cases where one twin is a very high achiever and the other not so, vs. where one twin is a very low achiever and the other not.

Finally, we acknowledge the complication caused by epigenetic modification of the genome. Genetically identical twins can vary in epigenetic processes such as methylation of cytosines within CpG dinucleotides and histone acetylation (Ollikainen et al., 2010; Carey, 2012) which can affect the expression of genes. Epigenetic differences may account for twin discordance in a variety of behavioral phenotypes (Petronis et al., 2003; Petronis, 2006), including, it is prudent to assume, academic achievement, though the current evidence suggests the effects are small. This possibility undermines strong claims that

MZ twin discordance signals the sole operation of non-molecular processes. That is, explanations of discordance as being due to factors such as the assignment of twins in a pair to a skilled vs. unskilled teacher have to compete with explanations that invoke differences between the twins in how their (identical) genes are expressed. Research on epigenetic factors in academic development is currently underway (Karlsson Linner et al., 2017), and future work may reveal how epigenetics and non-molecular factors play out in MZ twin discordance in education.

CONCLUSIONS

We have attempted to identify aspects of students' environments that affect achievement in literacy and numeracy in the middle school years. By using discordant monozygotic twins' data, we have controlled for genes and for family-based environmental factors such as SES, literacy environment, and parental attitudes to the value of mathematics. Measurement error, which in behavior-genetic studies is included in the unique environment term, can masquerade as genuine environmental influence, and we have attempted to minimize this threat by selecting twin pairs where the discordancy is substantial and in the same direction over at least three assessments spanning 5 calendar years.

We were able to assign environmental influences to one or more of three categories, biomedical, personal, and school-based. Biomedical factors could, in turn, be subdivided into conditions affecting one twin but not the other and conditions affecting one twin more severely than the other. There was an indication that the effect of a condition might be indirect, via adverse effects of a prescribed drug or via anesthesia at a young age. There was also evidence that a condition can be a stimulus to *higher* performance by encouraging compensating scholarly activity.

Personal factors included the somewhat overlapping (and relatively well-studied) categories of motivation and effort, enjoyment and interest, and personality characteristics such as conformity, risk aversion, and anxiety. Processes operating in the school setting included, not surprisingly, allocation to perceived higher- or lower-quality teachers, or to a poor teacher-student relationship. The influence of peers, for good or ill, also figured in parental reports. Academic streaming was seen as a consequence rather than a cause of twin discordancy for the most part.

Few of these factors operated in isolation, underlining the complexity of tracing the causal pathways of discordancy and consequently of environmentally-sourced influences on student performance. But we believe that qualitative investigations such as this one can contribute to clarifying these

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pathways and suggest avenues to follow with more rigorous, quantitative methods.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Ethics Approval for Research Involving Humans, required by the Human Research Ethics Committee within the University of New England with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Human Research Ethics Committee at the University of New England.

AUTHOR CONTRIBUTIONS

CH, BB, WC, and AS conceived the study design. BB, WC, SL, CH, and CL collected the interview data and contributed to initial thematic development. SL conducted the data analysis. SL and BB wrote the original manuscript. All authors contributed to revision and editing.

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SUPPLEMENTARY MATERIAL

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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