American Journal on Intellectual and Developmental Disabilities Examining the Relationship among Parental Stress, Knowledge, and Family Empowerment for Latinx Parents of Children with Intellectual and Developmental Disabilities

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Abstract:	Latinx parents of children with intellectual and developmental disabilities (IDD) face unique challenges, including heightened stress, limited access to resources, and systemic barriers. This study explores the relationships among parental stress, knowledge of special education, and family empowerment using structural equation modeling (SEM). Data were collected from 96 Latinx parents participating in a culturally responsive advocacy training program. Results revealed that greater knowledge was associated with increased empowerment but also heightened stress, reflecting the dual-edged nature of knowledge acquisition. Empowerment, however, mitigated stress related to dysfunctional parent-child interactions and difficult child. These findings underscore the need for culturally responsive interventions that balance knowledge building with stress management, promoting resilience and empowerment among Latinx families navigating the complexities of raising children with IDD.			

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Abstract

Latinx parents of children with intellectual and developmental disabilities (IDD) face unique challenges, including heightened stress, limited access to resources, and systemic barriers. This study explores the relationships among parental stress, knowledge of special education, and family empowerment using structural equation modeling (SEM). Data were collected from 96 Latinx parents participating in a culturally responsive advocacy training program. Results revealed that greater knowledge was associated with increased empowerment but also heightened stress, reflecting the dual-edged nature of knowledge acquisition. Empowerment, however, mitigated stress related to dysfunctional parent-child interactions and difficult child. These findings underscore the need for culturally responsive interventions that balance knowledge building with stress management, promoting resilience and empowerment among Latinx families navigating the complexities of raising children with IDD.

Examining the Relationship among Parental Stress, Knowledge, and Family Empowerment for Latinx Parents of Children with Intellectual and Developmental Disabilities

Parents of children with intellectual and developmental disabilities (IDD) experience significantly higher levels of stress than those with children without IDD (Patton et al., 2018). These disparities are even more pronounced within Latinx families. Compared to white parents of children with IDD, Latinx parents report experiencing greater stress (Magaña et al., 2015). Furthermore, Latinx parents encounter additional challenges, including limited access to knowledge about special education (Burke, Rios, Garcia, et al., 2020) and lower levels of empowerment (i.e., the ability to advocate for and effect positive change for their child with a disability, Burke, Rossetti, et al., 2021). These factors—stress, knowledge, and empowerment are interconnected. For example, a qualitative study of 16 Latina mothers of children with autism revealed that a lack of special education knowledge contributed to heightened stress levels (Rios et al., 2020). The study also highlighted systemic barriers these mothers encountered in accessing special education resources, with language barriers representing a significant obstacle (Burke, Rossetti, et al., 2021).

Exploring stress, knowledge, and empowerment among Latinx families is essential. Latinx parents represent one of the fastest-growing minority populations in the United States (U.S. Census Bureau, 2020), yet they continue to face greater service disparities (Magaña et al., 2015). Identifying factors related to stress within this population is critical, as is understanding how stress, empowerment, and special education knowledge vary according to a child's specific disability. Parents of children with autism, for instance, experience significantly higher stress than parents of children with other types of IDD (Hayes & Watson, 2013). However, families of children with autism also tend to report feeling more empowered and knowledgeable about their rights, as evidenced by the higher frequency of due process hearings in this group (Burke & Goldman, 2015). Understanding these differences can help in tailoring support for families of children with IDD across various cultural and disability contexts.

In addition, cultural factors further compound the challenges faced by Latinx families. Research suggests that cultural norms, such as *respecto* (respect for authority figures) and *familismo* (a strong emphasis on family unity), may discourage parents from questioning educators or advocating assertively for their children (Lopez et al., 2001). These cultural dynamics, while rooted in values that emphasize harmony and deference, can inadvertently contribute to lower levels of empowerment when parents feel unprepared or intimidated in school settings. This highlights the need for culturally responsive interventions that not only provide knowledge but also empower parents to navigate systemic inequities confidently.

More specifically, parental stress, family empowerment, and knowledge are critical constructs in understanding the experiences and outcomes of families with children who have disabilities. These constructs have long been studied to inform interventions and policies aimed at supporting families, yet their measurement remains a complex and evolving area of research (Hong & Rios, 2024). Accurate measurement models are essential to capture the nuanced dimensionality of these constructs, as well as to ensure their applicability across diverse populations and contexts.

Much of the existing research on stress and empowerment among Latinx parents has focused narrowly on correlates without fully exploring the complex relationships among stress, knowledge, and empowerment (Rios & Burke, 2023). Understanding these interactions is critical for developing holistic approaches that address the root causes of stress and promote resilience. For instance, while increased knowledge about special education can empower parents, it may also introduce new stressors as they navigate the system's complexities and advocate for their child's needs (Burke & Hodapp, 2014). Similarly, empowerment itself may act as both a buffer against stress and a mediator in the relationship between knowledge and stress. Examining these dynamics can provide deeper insights into how to support Latinx families effectively. To address these gaps, this study explores the relationships among knowledge, parental stress, and family empowerment for Latinx parents of children with IDD through structural equation modeling analyses. Guided by the following research questions, the study aims to advance understanding of these critical constructs within a culturally specific framework: (a) What is the effect of knowledge on family empowerment and parental stress? And, (b) What is the effect of family empowerment on parental stress?

Methods

Participants

This study included 96 Latinx parents of children with intellectual and developmental disabilities (IDD) in the United States. Inclusionary criteria required participants to be: Latino/a parents of children with IDD and registered for an advocacy training program. Participants were excluded if they did not identify as Latino/a and were not enrolled in the parent advocacy program. Here, the term Latinx describes individuals either born in or with family origins from Latin America (Comas-Diaz, 2001; Olivos et al., 2010). Although data were collected from various centers across the U.S. over five years, only 96 families participated due to two main recruitment challenges: the relatively low population of Latinx parents with children with IDD, and the demanding period in which families were invited to participate. This period can be especially taxing for parents, as involvement in research may add to their burdens. Nevertheless, to address the study's research questions and employ the complex statistical model shown in Figure 1, we conducted the power test using the semTools package in R (Jorgensen et al., 2022)

to support the challenges posed by a small sample size. The power analysis revealed that the 96 sample size produced a power of .995. It was adequate for the statistical analyses carried out in this study.

Data Collection Procedures

Institutional Review Board (IRB) approval was first obtained. Participants were selected through purposeful sampling (Patton, 2002) and recruited via local and statewide agencies, community organizations (e.g., parent support groups, Latinx serving churches), and social media like Facebook. The recruitment strategy embraced *personalismo*, a culturally responsive approach focused on building *confianza* (trust) between families and professionals (Magaña, 2000). A bilingual Latina researcher fostered connections with Latinx parents of children with IDD through volunteer work with Latinx organizations and community-based research. Eligible participants then completed a survey about the advocacy training program, which was available in both English and Spanish but was completed entirely in Spanish by all participants. The advocacy program, covered in previous research (Burke, Rios, et al., 2020; Burke, Rossetti, et al., 2021; Rios et al., 2021, 2024), provided 12 hours of training on special education policy, non-adversarial advocacy, and empowerment, and was delivered in Spanish by native Spanish-speaking Latina instructors. Each participant received a \$20 stipend, and the first author entered survey data into SPSS (IBM Corp., 2013) for analysis.

Measures

Parenting Stress Index Scale-Short Form (PSI-SF, Abidin, 2012)

The Parenting Stress Index-Short Form (PSI-SF, Abidin, 2012) is a 36-item questionnaire using a 5-point Likert scale, from 1 (strongly disagree) to 5 (strongly agree), where higher scores indicate greater parental stress. It evaluates parenting stress in three main areas: The term,

Parental Distress (PD) refers to stress that is particularly connected to being a parent and feelings of overload; Parent–Child Dysfunctional Interaction (PCDI), which looks into stress brought on by the parent's feeling that their relationship with the child does not meet their expectations; and Difficult Child (DC), which measures stress associated with challenging child behaviors. The PSI-SF is available in Spanish (Solis & Abidin, 1991) and has shown high reliability when used with Latinx parents of children with IDD (e.g., Burke & Hodapp, 2014).

Family Empowerment Scale (FES, Koren et al., 1992)

The Family Empowerment Scale (FES) assesses empowerment across three areas: family, services, and community (Koren et al., 1992). The Family Subscale evaluates a parent's involvement in their child's life, while the Services Subscale focuses on the parent's sense of empowerment related to disability services for their child. The Community Subscale measures the parent's engagement in various community activities. For example, participants respond to items such as, "I feel that I have the right to approve all services my child receives." Responses are rated on a 5-point Likert scale, with higher scores reflecting greater empowerment. The FES has demonstrated strong reliability when used with Spanish-speaking parents of children with intellectual and developmental disabilities, with subscale alphas of 0.93, 0.89, and 0.85 (Canino et al., 2008).

Special Education Knowledge (Burke et al., 2016)

Comprised of 10 multiple choice items about special education knowledge, this scale has reliability with parents of children with IDD, including Spanish-speaking, Latinx families of children with IDD (e.g., using the dichotomous items [0 = incorrect, 1 = correct]).

Data Analysis

We conducted structural equation modeling analysis in the *lavaan* package (Rosseel, 2012) in R (R Core Team, 2024) and Mplus 8.10 (Muthén & Muthén, 1998-2017) using 96 Latinx parents of children with intellectual and developmental disabilities who responded to the Family Empowerment Scale, Parental Stress Index-Short Form (PSI-SF), and Knowledge scale. Goodness of fit was evaluated by using the comparative fit index (CFI), Tucker Lewis index (TLI), and root mean square error of approximation (RMSEA). Models are considered to be adequate fits if CFI and TLI are larger than or equal to 0.90 and RMSEA is smaller than 0.08 (Hu & Bentler, 1999). Notably, no data were missing.

Sample Characteristics

The demographics of the 96 parents are shown in Table 1. 97% (n = 93) of the individuals in the study were female, and their average age was 40.854 years (SD = 6.852). With an average age of 9.5625 (SD = 4.717), 58.33% (n = 56) of the children were 9 years of age or less, and the majority of the children were male (77.1%, n = 74). 43.8 percent (n = 42) of parents who self-reported their child's disability reported their child had autism. The majority of families (85%, n = 82) earned less than \$49,000 per year. Of the parents, most claimed having some college education (22.9%, n = 22), graduating from high school (31%, n = 30), and having some high school education (28.1%, n = 27). Just 17.7% (n = 17) reported having a graduate or four-year degree.

Results

Descriptive Statistics

Table 2 presents the average scores and standard deviations of PSI, PD, PCDI, DC, family empowerment, and knowledge for all 96 Latinx parents at both the total and item levels. Pearson correlation matrices are displayed for the items by each dimension of PSI, FES, and Knowledge in Table 3. The correlation between empowerment, knowledge, and parental stress ranges from - 0.016 to 0.451. While the items between PD, PCDI, and DC are highly correlated, items are weakly or moderately related between empowerment, knowledge, and parental stress. The assumption of normality was assessed using the Shapiro-Wilk test. Results indicated that the data were normally distributed (W = 0.97, p = 0.06).

Measurement Models

Parental Stress, Family Empowerment, and Knowledge

Measurement models of parental stress, family empowerment, and knowledge were assessed as follows: First, the conventional reliability estimates (alpha and omega) were assessed as shown in Table 2. Both Cronbach's alpha and omega (often McDonald's omega, 1999) are measures of internal consistency reliability, which assesses how well a set of items (like questions on a survey) measure the same underlying construct. Cronbach's alpha estimates reliability based on the average inter-item correlations (Cronbach, 1951). However, it is often inflated by a large number of items and can be misleading if item variances differ a lot. McDonald's Omega (McDonald, 1999) is considered a better estimate of the proportion of variance in total scores that is due to the general factor. Omega is more flexible and accurate when items differ in how well they reflect the latent trait and less sensitive to the number of items or their uniformity (Hayes & Coutts, 2021; McDonald, 1999; McNeish, 2018). With the exception of knowledge, which has alpha and omega reliability values of .639 and.650, respectively, all of the scales have high average alpha and omega coefficients, ranging from .850 to.961.

Next, based on prior theory (Hong & Rios, 2024) pertaining to parental stress, the dimensionality was evaluated with a bifactor confirmatory factor analysis model with weighted

least squares with mean and variance adjusted (WLSMV) estimation. The bifactor model consists of a general factor of total parental stress (PSI) and group factors of PD, PCDI, and DC (See Figure 1). Second, the dimensionality of family empowerment was assessed using both three-factor and bifactor confirmatory factor analysis model with WLSMV estimation, which was based on previous theory (Koren, Dechillo, & Friesen, 1992). The three-factor model comprises factors of family, service, and community while the bifactor model includes a general factor of overall family empowerment as well as group factors of family, service, and community (See Figures 2 and 3). Finally, a one-factor confirmatory factor analysis model with WLSMV, based on earlier theory (Dunst et al., 1988), was used to evaluate the dimensionality of knowledge (See Figure 4).

Model fit statistics reported in Table 4 includes the obtained model χ^2 , its degrees of freedom, CFI, or Comparative Fit Index, TLI, or Tucker Luis Index (in which values higher than .90 are desirable for adequate fit), and the RMSEA, or Root Mean Square Error of Approximation, (in which values lower than .08 are desirable for a good fit). Following the guidelines of acceptable model fit provided by Hu and Bentler (1999), each of the overall goodness-of-fit indices suggested that the bi-factor model fit of parental stress the data adequately: x^2 (558) = 895.78, RMSEA = 0.079, CFI = 0.918. However, the three-factor model fit of family empowerment was not good: x^2 (524) = 1155.373, RMSEA = 0.113, CFI = 0.863. Applying the bifactor model improved the model fit, as Table 4 indicated: x^2 (490) = 824.283, RMSEA = 0.084, CFI = 0.929. The one-factor model for the knowledge scale revealed that the model fit was excellent (See Table 4).

Structural Model

The structural model examined the relationships among the latent factors of

empowerment (family, service, and community), parental stress (parental distress, parent-child dysfunctional interaction, and difficult child), and knowledge. Table 5 shows the results of structural equation modeling analyses. This bifactor model, as shown in Figure 1, achieved the adequate fit, $\chi 2(474) = 3510.539$, CFI = .918, TLI = .913, RMSEA = .042.

The Effect of Knowledge on Empowerment

Our findings indicated that knowledge is positively related to parents' empowerment. In other words, the more knowledge the parents possess, the more they feel empowered to possess a heightened sense of agency and control (b = .900, p <.001).

The Effects of Knowledge on Parental Stress

Knowledge is also positively associated with parental distress (PD), parental-child dysfunctional interaction (PCDI), and Difficult Child (DC). It is intriguing to note that parents with greater expertise exhibit higher levels of PD, PCDI, and DC.

The Effect of Empowerment on Parental Stress

Finally, empowerment is negatively related to parental-child dysfunctional interaction (PCDI) and perceptions of the child as a difficult child (DC). In other words, less empowered parents tend to have higher PCDI (b = -1.980, p < .05) and DC stress (b = -1.093, p < .01). A follow-up analysis was conducted to include the covariates of age, disability type, education level, and income but these covariates were found to be non-significant.

Discussion

This study produced three main findings. First, knowledge was positively related to parents' empowerment. Empowerment, in this context, reflects parents' confidence in making decisions and advocating for their children effectively. This aligns with prior research indicating that knowledge can serve as a critical component of empowerment by enhancing parents' selfefficacy and reducing feelings of helplessness (Koren et al., 1992). Furthermore, knowledge equips parents with the tools to navigate systems such as special education and healthcare, fostering a sense of agency and control over their child's outcomes (Dempsey & Dunst, 2004).

Second, knowledge was positively associated with all three subscales of parental stress. This may seem counterintuitive; however, it reflects findings from earlier studies suggesting that increased awareness can initially heighten stress levels, as parents become more cognizant of the challenges their child may face (Hassall et al., 2005). This highlights the dual-edged nature of knowledge—it empowers parents but can also burden them with greater emotional and practical responsibilities. Notably, some research suggests that gaining knowledge about special education and child-related challenges can initially lead to heightened stress among parents. This phenomenon occurs because increased awareness often brings greater recognition of the difficulties and systemic barriers that need to be addressed. For example, Hassall, Rose, and McDonald (2005) found that parental cognitions, particularly those related to a child's needs and the required level of support, were positively correlated with stress levels. Similarly, Green (2007) noted that as parents become more informed about their child's condition and the special education process, they often experience an initial spike in stress due to the realization of the complexity of advocating for their child.

Lastly, empowerment is negatively related to parental-child dysfunctional interaction (PCDI) and perceptions of the child as difficult (DC). Less empowered parents have higher stress, which means that intervention can help empower parents. This supports the theory that empowered parents are better equipped to manage their child's behavior and engage in positive interactions. Empowerment can mitigate feelings of frustration and help parents reframe their

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child's challenges more constructively (Zimmerman, 1995). Consequently, this finding underscores the critical role of empowerment as a protective factor against dysfunctional parentchild dynamics.

Limitations

Several limitations were found during the study's execution, which could have an impact on how the findings are interpreted. Frist, even if the power analysis indicated that the sample size of 96 achieved the power of 0.995, it is not advisable to make general statistical inferences from this study due to its tiny sample size. Despite being a critical choice in SEM, there is little consensus in the literature over what an appropriate sample size is. Although there is evidence that basic SEM models could be usefully tested with relatively small sample sizes (Hoyle, 1999; Hoyle & Kenny, 1999; Marsh & Hau, 1999), the minimum sample size normally required to perform SEM is believed to be between 100 and 150 (Anderson & Gerbing, 1988; Ding, Velicer, & Harlow, 1995; Tinsley & Tinsley, 1987). Second, since data are collected at one point in time, we can observe the relationships between variables, which cannot establish cause and effect. Future research must further address temporal dynamics and developmental trajectories, as the cross-sectional character of the study precludes the inference of causal links between variables. Third, the normal caregiving responsibilities that women do, especially in Latinx households with children with IDD, are probably the reason why women are overrepresented in our sample. Women's care may be considered advantageous as, as mothers, they may be the ones who are most familiar with their children's IDD needs. However, the stress levels of these parents might be misrepresented by gender-specific sampling. Therefore, care should be taken while interpreting the results. It could be necessary for future research to devise a plan for aggressively enlisting male subjects. Finally, the PSI-SF, FES, and Knowledge scales were self-reported by Latinx parents. Participants may answer in a way they think looks good or is socially acceptable,

rather than being fully honest. The reliance on self-reporting introduces potential biases such as social desirability, recall inaccuracies, and subjective interpretation, which may affect the reliability and validity of the findings. Recognizing these restrictions is critical for contextualizing our findings and guiding future research.

Implications for Research

The results of this study provide various implications for future studies. First, the highly reliability estimates for the majority of scales support their continued use in research and practice, while the lower reliability of the knowledge scale suggests the need for refinement to enhance its internal consistency. Future research should explore potential cultural or contextual factors that may influence these scales' psychometric properties, particularly for knowledge. Additionally, the descriptive statistics highlight the importance of capturing both total and item-level scores to fully understand their implications. Thus, if replicated, future research should be conducted with a larger sample size to ensure that these findings are consistent across other groups of participants.

In addition, the findings on measurement models suggest opportunities for advancing theory and practice in understanding the constructs of parental stress, empowerment, and knowledge. While the bifactor model of parental stress demonstrated adequate fit, highlighting the multifaceted nature of stress, the initial three-factor model of family empowerment did not fit well, prompting the use of a bifactor model to improve its utility. These results suggest that future research studies should utilize more nuanced modeling approaches that may better capture the complexities of empowerment. For knowledge, the excellent model fit reinforces its theoretical grounding but also points to the need for research investigating its role across diverse cultural groups to generalize the findings. Researchers should further test these models in broader populations and explore the interplay among these constructs to refine intervention strategies.

Implications for Practice

The study's findings emphasize the importance of developing and implementing culturally responsive practices to support Latinx parents of children with IDD. The strong association between knowledge and empowerment highlights the need for targeted educational interventions that provide parents with information and resources tailored to their cultural and linguistic needs. Practitioners should prioritize workshops, informational sessions, and accessible materials that address the unique challenges faced by these families. However, given the positive relationship between knowledge and stress, practitioners must also ensure that knowledgebuilding efforts coupled with emotional support and stress management resources help parents navigate the complexities of caregiving with confidence and resilience.

In addition, the negative relationship between empowerment and stress, such as parentchild dysfunctional interactions and perceptions of the child as difficult, emphasizes the critical role of empowerment-focused interventions. Professionals working with Latinx families should consider strategies that foster parental agency and self-efficacy, such as strengths-based coaching, family-centered planning, and opportunities for parents to advocate within their communities. By helping parents feel more in control and capable, these approaches can reduce stress and promote healthier family dynamics. Notably, the findings support the utility of comprehensive assessment tools to identify specific areas of stress and empowerment, guiding practitioners in creating individualized, culturally competent intervention programs.

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Sample Characteristics (N = 96)

Chara	Percent (Frequency) or Mean (SD)	
Gender: Female		96.9% (93)
Education background	Some high school	28.1% (27)
	High School Degree	31.3% (30)
	Some college	22.9% (22)
	4 year degree	8.3% (8)
	Graduate/Professional Degree	9.4% (9)
Annual household income	Less than \$15,000	30.2% (29)
	Between \$15 and 29,000	32.3% (31)
	Between \$30 and \$49,000	22.9% (22)
	Between \$50 and \$69,000	7.3% (7)
	Between \$70 and \$99,000	6.3% (6)
	Over \$100,000	1% (1)
Ethnicity	Mexican	85.4% (82)
	Puerto Rican	4.2% (4)
	Central American	4.2% (4)
	South American	5.2% (5)
	Other-mixed	1% (1)
Child disability	Autism	43.8% (42)
	Other	56.2% (54)
Child gender	Male	77.1% (74)
	Child age	9.56 (4.72)

Scale	Mean: Scale (Item)	SD: Scale (Item)	Alpha	Omega	Range
PSI	116.385 (3.233)	25.133 (.698)	0.931	0.934	1-5
PD	37.167 (3.097)	10.390 (.866)	0.897	0.904	1-5
PCDI	40.083 (3.34)	10.032 (.836)	0.857	0.866	1-5
DC	39.135 (3.261)	9.367 (.781)	0.85	0.856	1-5
Empowerment	114.74 (3.375)	25.516 (.750)	0.949	0.961	1-5
Knowledge	2,375 (.238)	2,079 (208)	0.639	0.65	0 or 1

Descriptive Statistics

Knowledge2.375 (.238)2.079 (.208)0.6390.650 or 1Note. PSI: Parenting Stress Index; PD: Parental Distress; PCDI: parent-child dysfunctionalinteraction; DC: difficult child.

	Empowerment	PD	PCDI	DC	Knowledge
Empowerment	1	0.19 6	0.211	$0.\overline{05}$ 7	0.451
PD	-	1	0.873	0.67 6	0.310
PCDI	-	-	1	0.80 2	0.265
DC	-	-	-	1	-0.016
Knowledge	-	-	-	-	1

Note. PSI: Parenting Stress Index; PD: Parental Distress; PCDI: parent–child dysfunctional interaction; DC: difficult child.

	Number of items	Number of parameters	Scaled Chi- Square	DF	CFI	TLI	RMSEA
PSI : Bifactor	36	218	895.78 3	558	0.918	0.907	0.079
FES: Three factor	34	186	1166.3 73	524	0.863	0.854	0.113
FES: Bifactor	34	220	824.28 3	490	0.929	0.919	0.084
Knowledge: One factor	10	35	32.439	35	1	1	0

Model Fit Statistics of Parental Stress, Family Empowerment, and Knowledge

Note. PSI: Parenting Stress Index; FES: Family Empowerment Scale; Knowledge: Special Education Knowledge.

Structural Models of the Effects of Knowledge on Family Empowerment and Parental Stress and

Predictor (Independent Variable)	Outcome (Dependent Variable)	Main Effect		
	Total Empowerment	.900***		
Knowladza	PD	1.126**		
Knowledge	PCDI	2.275**		
	DC	.841*		
	PD	900		
Total Empowerment	PCDI	-1.980*		
	DC	-1.093**		

the Effects of Family Empowerment on Parental Stress



Note. PSI: Parenting Stress Index; PD: Parental Distress; PCDI: parent–child dysfunctional interaction; DC: difficult child.

Bifactor Model of Parenting Stress Index

Three Factor Model of Family Empowerment Scale



Note. Fml: Family; Srv: Service; Cmm: Community

Bifactor Model of Family Empowerment Scale



Note. Emp: Empowerment; Fml: Family; Srv: Service; Cmm: Community

One Factor Model of Special Education Knowledge



Note. knw: Special Education Knowledge

Structural Model Depicting the Significant Relationships among Knowledge, Family

Empowerment, and Parental Stress



Note. PD: Parental Distress; PCDI: parent–child dysfunctional interaction; DC: difficult child. *p < 0.05. **p < 0.01, ***p < 0.001.