

The Longitudinal Israeli Study of Twins (LIST)—An Integrative View of Social Development

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The Longitudinal Israeli Study of Twins (LIST) is a social developmental study, which implements social-developmental, molecular genetic, epigenetic, and behavioral genetic methods to advance knowledge on the development of individual differences in social behavior. Twins are followed from the age of three and both observational and parental-questionnaire data are collected on their empathy, temperament, and pro-social behavior. The parenting styles of parents are also evaluated using self-reports and observations and DNA samples are collected from parents and twins. In the current paper, we provide a review of our recent work and discuss the future aims of the LIST.

■ **Keywords:** LIST, behavioral genetics, pro-social behavior, parenting, temperament

Since behavioral genetic research established the influence of both environmental and genetic influences on a spectrum of traits and behaviors (Plomin et al., 2008), the age-old question of nature versus nurture has been, to a great extent, replaced or extended by questions regarding the specific genetic and environmental effects involved in each trait/behavior. This advancement opened the door to research on the nature of gene-environment interactions (GxE; e.g., risk vs. differential susceptibility, and possibility of resilience to environmental influences; Caspi et al., 2003; Ellis et al., 2011), and on potential epigenetic mediators of these interactions (McGowan et al., 2009). Similarly, more attention has been drawn to gene-environment correlations (rGE; Plomin et al., 1977) as an important interplay of genetic and environmental influences (Jaffee & Price, 2007). These progressively more complex questions require a broader coverage of phenotypes, genes, and environments, nested in a longitudinal design, in order to understand development. With these questions in mind the Longitudinal Israeli Study of Twins (LIST) was designed (Knafo, 2006).

The LIST aims to advance knowledge on the development of individual differences in children's social behavior, using a comprehensive cognitive-developmental-social-genetic design. The LIST is structured to follow children and their parents every two years or less from the age of 3 years, and to collect four types of data: questionnaire, observational, physiological (added at the age of 7 years), and genetic (some epigenetic data are also available on a

sub-sample). The scope of the data enables an investigation of rGE, GxE, and the crossroads of genes and environment (epigenetics).

Data Collection

The Ministry of the Interior has provided data on families of monozygotic (MZ) and dizygotic (DZ) twins. When the twins reached the age of 3 years, questionnaires were sent to the families, which included questions on the pregnancy, twins' behavior—particularly pro-social behavior—twins' characteristics, the relationship between the twins, demographic details, socioeconomic status, and questions regarding the twins' zygosity. At ages 3.5, 5, 6, and 7 years, families living in Jerusalem and nearby surroundings were invited to partake in an experimental session at the laboratory (e.g., Avinun, Ebstein, & Knafo, 2012; Pener-Tessler et al., 2013 Kavé, Shalmon, & Knafo, in press). At the age of 6 years, we were also able to cover additional areas in

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TABLE 1
LIST MZ and DZ Pairs and Data Collected According to Age and Data Type

	Observational/test data				Parental questionnaire data			
	3.5	5	6	7	3	5	6	7
Participants								
MZ	63	50	160	38	302	195	143	23
DZ same-sex	93	85	313	48	556	404	291	30
DZ opposite-sex	67	60	71	22	535	354	66	15
Study variables								
Temperament			✓		✓	✓	✓	✓
Psychological problems					✓	✓	✓	✓
Pro-social behavior	✓	✓	✓	✓	✓	✓	✓	✓
Empathy	✓	✓	✓	✓	✓	✓	✓	✓
Cognitive abilities	✓	✓	✓					
Sociocognitive abilities	✓	✓		✓				
Maternal behavior	✓		✓	✓	✓	✓	✓	✓
Paternal behavior			✓		✓	✓	✓	
Twin relationships	✓	✓	✓	✓	✓	✓	✓	✓
Values				✓				
Medical history					✓	✓	✓	✓
Demographic information					✓	✓	✓	✓

Note: Participant numbers do not include pairs whose zygosity has not been verified or estimated yet. Data collection is ongoing.

Israel and conduct the experiment at families' homes when necessary.

Table 1 depicts sample sizes for questionnaire and observational data separately according to age and zygosity. Thus far data have been collected from 1,636 twin families, 19.7% MZ twins, 71.1% DZ twins, and 9.2% of unknown zygosity. The high proportion of DZ twins is due to the relatively high number of Israeli families who use assisted reproductive technologies. DNA data collection is ongoing and at the time of this report is available on 37.7% of the sample. Zygosity is assigned based on parental questionnaire data (Goldsmith, 1991) or 10 genetic polymorphisms when DNA data are available. As found in previous research (Price et al., 2000), the algorithm used to assign zygosity with a questionnaire method was in 95% agreement with DNA results.

Behavioral measurements

Table 1 also describes the types of data that have been collected (for further details see Knafo, 2006). As can be seen, data on pro-social behavior and empathy, two key variables in LIST, have been collected at each time point using both questionnaire and observational methods. Other measured attributes and characteristics, in addition to their stand-alone importance, have been linked to children's social behavior and were therefore included in LIST [e.g. parenting (see Knafo & Plomin, 2006a); cognitive ability (see Zahn-Waxler et al., 1982); and temperament (see Russell et al., 2003)].

Genetic data

Since the focus of LIST is social, and particularly pro-social behavior, there are three neurobiological systems of special interest: dopaminergic, serotonergic, and oxytonergic\vasopressinergic. Dopaminergic brain areas are associated with reward (Beninger & Miller, 1998; Girault & Greengard, 2004; Schultz, 2010), and have been shown to be activated by decisions to donate to charities, thus suggesting their role in the 'warm glow' feeling that promotes other-oriented\pro-social behaviors (Moll et al., 2006). Serotonin has been associated with various characteristics related to social behavior such as decision-making (Stoltenberg & Vandever, 2010), aggression (Kuepper et al., 2010), and neuroticism (Munafò et al., 2006). The oxytocin and vasopressin nonapeptides have also been associated with social behavior in various species (Donaldson & Young, 2008; Ebstein et al., 2010). Thus, main candidate genes for our molecular genetic studies include the dopamine D4 receptor gene (*DRD4*), the dopamine D5 receptor gene, the serotonin transporter gene, the catechol-O-methyltransferase gene, the arginine vasopressin receptor 1A gene (*AVPR1A*), and the oxytocin receptor gene.

Recent Major Achievements in LIST

The first phases of LIST (ages 3–5 years) focused on empathy and on pro-social behavior, defined as a voluntary act aimed at benefiting others (Eisenberg et al., 2006). The three main forms of pro-sociality, namely sharing, caring, and helping, can all be initiated in response to internal or external motivations (self-initiated or compliant pro-social behavior, respectively). Self-initiated pro-social behavior can be seen as more advanced in terms of cognitive development as it requires going one step further than conforming to schematic or social demands (Knafo et al., 2011b).

In accordance with previous research (Eisenberg et al., 1984) our results from age 3.5 years have shown that self-initiated and compliant pro-social behavior do not intercorrelate (Knafo et al., 2011a), implying these behaviors represent inherently different constructs. In the same study, it was also shown that genetic influences account for a substantial proportion of variance in observed self-initiated (43%) and compliant pro-social behavior (34%) (Knafo et al., 2011a). The rest of the variance was accounted for by non-shared environment. We found a similar pattern with observed empathy (Knafo et al., 2009). These results join the young but growing literature showing that genetic factors contribute to children's and adolescents' pro-sociality (Gregory et al., 2009; Hur & Rushton, 2007; Knafo & Israel, 2009; Knafo & Plomin, 2006b; Knafo et al., 2009; Scourfield et al., 2004; Volbrecht et al., 2007; Zahn-Waxler et al., 2001; but also see van IJzendoorn et al., 2010).

In LIST, the influence of maternal behavior was examined on compliant and self-initiated pro-social behavior (Knafo et al., 2011a). Maternal positivity, negativity,

and unreasoned punishment did not correlate significantly with preschoolers' pro-social behavior. However, a gene-environment interaction was found with variation in the exon III repeat region of the *DRD4* gene. Only the pro-social behavior of children, who were carriers of the 7-repeat allele, was correlated with mothers' self-reported parenting (Knafo et al., 2011a). A similar pattern was seen with children's empathic concern (Knafo & Uzefovsky, in press), a vicarious emotional response to others, which is closely linked to pro-social behavior. Maternal negativity related negatively to observed empathy toward an examiner at 3.5 years, but only among children carrying the 7-repeat allele.

Interestingly, as further shown by our group (Fortuna et al., 2011b), the same seems to hold true when the roles are reversed, with mothers as the *DRD4* 7-repeat allele carriers and the children as their surrounding environment. Higher levels of child medical risk at birth (e.g., gestational age at birth, birth weight, and length of stay at the neonatal intensive care unit) were associated with less sensitive parenting only among mothers carrying the 7-repeat allele. Moreover, mothers who were carriers of the 7-repeat allele and whose children scored low on the risk index were observed to have the highest levels of sensitivity. These results support previous research (Ellis et al., 2011) showing that the 7-repeat allele is a differential susceptibility allele, moderating the way the environment influences behavior, for better or for worse. Birth weight, which, as mentioned, is an index of medical risk, was also shown to be a predictor of children's conduct problems in discordant twins (Mankuta et al., 2010).

The data collected in LIST also enabled us to be the first to associate preschoolers' altruistic behavior with a variant of the RS3 polymorphism in *AVPR1A* (Avinun et al., 2011), which was previously associated with autism (Kim et al., 2002), and to associate altruism in adults with variation in the oxytocin receptor gene (Israel et al., 2009).

Peer relationships are also a key factor in the acquisition of social skills and traits, to the point that they were claimed to have a larger influence than parenting (Harris, 1998). We investigated the relationships between children's peer problems and three temperamental dimensions: negative emotionality (an individual's tendency to experience distress), sociability (an individual's ability to enjoy interpersonal contexts), and activity level. Results showed that children characterized by negative emotionality and low sociability and activity level tended to have greater peer problems. Moreover, bivariate genetic analyses showed that most of the relationship between temperament and peer problems was due to overlapping genetic factors which contributed to both traits (Benish-Weisman et al., 2010). Interestingly, a mirror image of this pattern was found with regard to pro-social behavior, which related negatively to negative emotionality and positively to activity level and sociability (Knafo & Israel, 2012).

The study of twin relationships may have important implications not only for understanding the interesting social dynamics of this growing group of children, but also for the assumptions and generalizability of behavioral genetic research, and yet it remains relatively scarce. A comparison between maternal reports of 3-year-old DZ and MZ twins' rivalry, conflict, closeness, and dependence, has shown that MZ twins (including those mistakenly considered as DZ by their mothers) were perceived as closer and more co-twin dependent relative to DZ twins. Same-sex twins were reported to have lower dependence and higher levels of conflict and rivalry than opposite-sex dyads. In addition, as compared with a matched sample of non-twin siblings, DZ twins were described as closer and more co-dependent (Fortuna et al., 2011a). These findings lend support to theories regarding the effects of genetic similarity and gender in shaping relationships, and speak to the special nature of twins' relationships.

Future Directions

The longitudinal aspect of the collected data will enable us to examine stability and change throughout development in various pro-social, temperamental, and parental characteristics. The second phase of LIST (ages 6–7 years, and a planned age 8–9 wave) has two main goals. The first is to provide an in-depth investigation of gene–environment correlations, a subject that has received very little attention (Knafo & Jaffee, 2013). We will rely on the inclusion of parental behavior in the study design and the collection of DNA from parents and twins (e.g., Pender-Tessler et al., 2013). A first report from LIST found evidence for evocative gene–environment correlations, indicated by heritability of observed maternal behavior, in mothers' intrusiveness but not in their warmth (Knafo, 2011).

The second main goal is to further understand pro-social development. For example, we are currently investigating the role of children's values in the development of pro-social and empathic behaviors, and their association with physiological biomarkers of the autonomic nervous system. In addition to studying potential phenotypic relationships, we will investigate the role of genetics in these relationships as well as parent-child cycles of influence.

Finally, in addition to the effects of the candidate genes mentioned above, we are interested in how gene–environment interactions manifest in epigenetic changes in the genome. The collection of genetic, epigenetic, biological, and environmental data is aimed at providing an integrated view of the developmental processes underlying social behavior.

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