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**Project Title:** Intra-Genomic Conflicts and Social Decision-Making in Humans
**Site of Research:** Department of Psychology, Åbo Akademi University (ÅAU), 36 months
**Date of Research Plan:** September 2015 (The current plan is an adapted version of my plan in 2014)

## 2 Rationale

Humans possess two homologous sets of chromosomes, and each allele inherited from one parent is matched with an allele inherited from the other parent. By epigenetic processes an allele can "remember" its parental origin and show parent-specific gene expression[1]. This remarkable phenomenon, called genomic imprinting (GI), means that alleles can be expressed *differently* depending on whether they have been inherited from an individual's mother or father. Imprinted alleles can be expressed when inherited from the father and silenced when inherited from the mother (paternally expressed; $P_{EXP}$) or expressed when inherited from the mother and silenced when inherited from the father (maternally expressed; $M_{EXP}$) (Fig 1, Panel A).

GI has profound implications on evolutionary explanations of social behavior[2–5], but until now no direct tests of this theory in humans exist. In the proposed research I will provide the first experimental tests on GI and social behavior in humans.

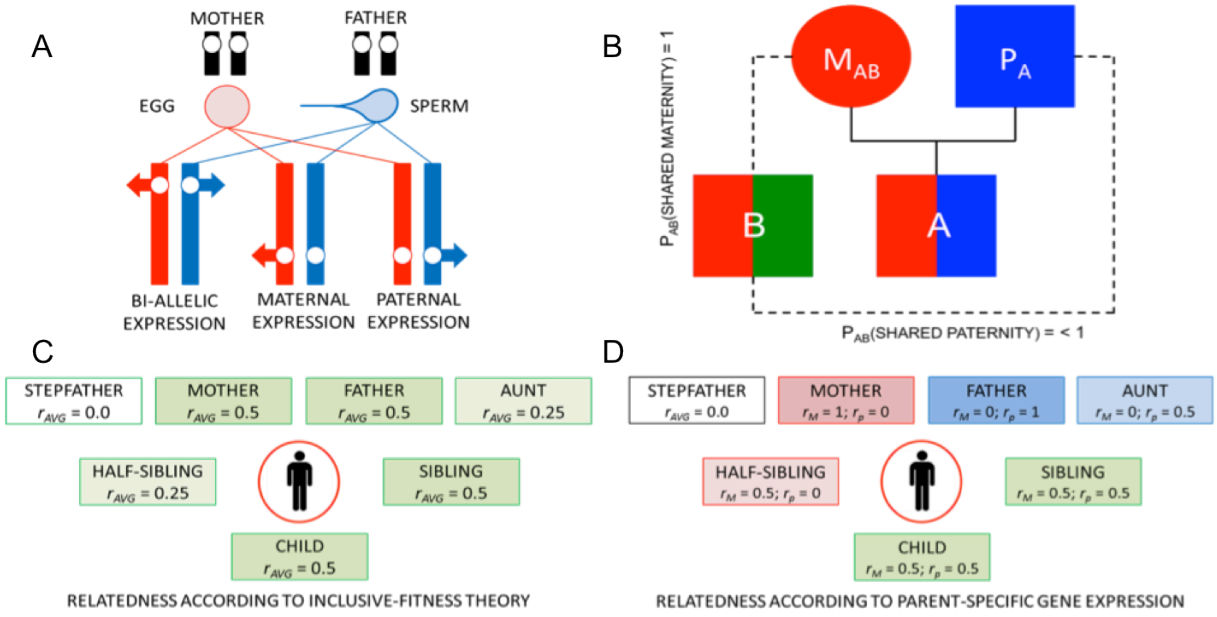



*Fig 1*. (**Panel A**) Genomic imprinting (GI): epigenetic processes in the gametes allow alleles to be expressed differently depending on whether they are inherited from the father or the mother. If one parental allele is silenced, only the allele inherited from the other parent is expressed. When both parental alleles are expressed (bi-allelic expression), a $P_{EXP}$ allele and a $M_{EXP}$ allele can work antagonistically, motivating diametrically different phenotypes. (**Panel B**) Because women can have offspring with multiple partners, the probability that a $P_{EXP}$ allele in an offspring (A) is present in a maternal sibling (B) is smaller than that of a $M_{EXP}$ allele. This difference in probabilities means that $P_{EXP}$ alleles benefit from exhausting maternal resources while $M_{EXP}$ alleles benefit from balancing maternal investment between all her offspring. Likewise, $P_{EXP}$ alleles benefit from a selfish and non-altruistic disposition towards siblings (especially under conditions where the probability of shared paternity is low). Again, $M_{EXP}$ alleles, which are equally likely to be present in her children A and B, benefit from dividing resources more equally, motivating a more altruistic behavior towards siblings. (**Panel C**) Inclusive-fitness theory gives that from a focal person's viewpoint 50% of his/her genetic material is shared with a mother, a father, a child, and a full-sibling. With a half-sibling 25% and with a stepfather 0% of the genetic material is shared. (**Panel D**) GI separates between paternal and maternal degrees of relatedness. From the same focal person's viewpoint, both $P_{EXP}$ and $M_{EXP}$ alleles are shared only with full siblings, and with one's own children (i.e., symmetric kin; marked with green). All other family members (half-siblings [including half-siblings that are mistakenly believed to be full-siblings], the mother, the father, aunts, uncles, nieces, and nephews) are either maternal or paternal (i.e. asymmetric kin; marked with red [maternal] and blue [paternal]).

Evolutionary explanations of social behaviors (i.e., all behaviors affecting both the actor and a recipient, such as altruism[a]) have until recently rested firmly on inclusive-fitness theory (IFT[6]). As explained by IFT, altruistic predispositions towards close kin can evolve when they are less costly to the actor than they are beneficial to the recipient. The degree of relatedness between the actor and the recipient scales this relationship. If the recipient is a full-sibling of the actor, the benefit of the act to the sibling needs to be twice as high as the cost to the actor as there is only a 50% chance that the allele motivating the altruistic disposition in the actor is also present in the sibling. If one considers a half-sibling, with which the actor shares 25% of alleles, IFT suggests that the benefit needs to be at least four times larger. (Fig 1, Panel C). Also other social behaviors can be explained by IFT: A disposition to avoid detrimental behaviors can evolve as a result of kin-selection. One example is inbreeding avoidance. Inbreeding decreases heterozygosity in the genome. This increases the risk of harmful recessive alleles being expressed, which in turn leads to a dramatically heightened risk of death or developmental problems in the offspring[7,8]. Inbreeding does not only produce evolutionary costs to an actor but also to his/her relatives (they also share their genetic material with the inbred offspring)[9]. Together with my colleagues, I have shown that patterns of inbreeding avoidance in humans can be largely explained by IFT. In this study, 2,353 participants conducted 27,364 forced-choices between various scenarios describing sex between two members of their family. Both first-person scenarios (i.e., the participant was described as having sex with a relative) and third-person scenarios (i.e., a sibling to the participant was described as having sex with a relative) were included. Relatives were selected so that each scenario varied both in terms of how much more harmful (i.e., how closely related the relatives where) one scenario was (vs. other scenarios) to the successful propagation of the participants alleles, and in terms of whether these costs are direct or obtained indirectly, only via relatives. The difference in the fitness cost between the scenarios—estimated from IFT—was highly correlated with the probability of a scenario being chosen as more aversive than its pair ($r = .913$, $p < .001$). Moreover, there was *no* effect of whether these costs were direct or indirect ($p = .780$), providing strong support to the notion that humans compute the inclusive-fitness costs of inbreeding and that this mechanism benefits the transmission of our *alleles* irrespective of our self-involvement. (Fig 2)

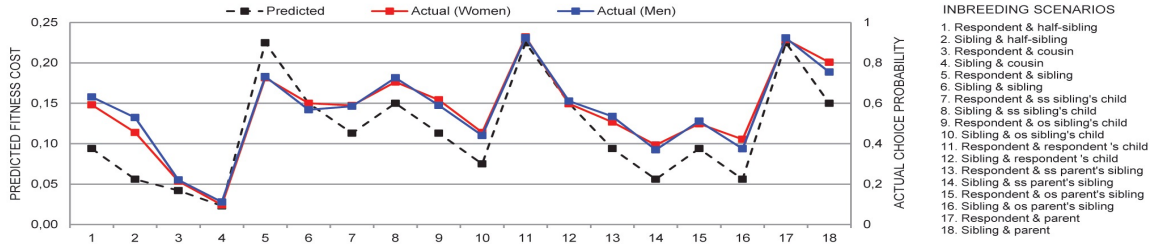



*Fig 2*. Fitness decrease by inbreeding-type (predicted values; y-axis left) and the probability of choosing an inbreeding-type as less preferred than its alternative (observed values; y-axis right) in a PC paradigm. Choice probabilities for women (red line) and men (blue line) displayed separately.

In the proposed research I will expand on my prior work on using decision-making paradigms to measure patterns of kin-directed behavior as a function of evolutionary theory. Although the explanatory power of IFT is substantial, GI adds considerable complexity to this standard view of the human family. GI suggests that most of our family relationships are characterized by an internal evolutionary conflict[5,10]. IFT averages relatedness across both paternally and maternally inherited alleles, whereas GI shows that maternal and paternal relatedness needs to be considered separately. While an individual, on average, shares 50% of her allele copies with any one of her parents ($r_{AVG} = 0.5$), a maternally inherited allele is always present in the mother but not in the

[a] While altruism is the standard example of a social behavior, the list of social behaviors also includes behaviors (and their absence) ranging from inbreeding and co-operation to breast-feeding and social smiling.

father ($r_M$ = 1.0 and $r_P$ = 0.0) and a paternally inherited allele is always present in the father but not in the mother ($r_M$ = 0.0 and $r_P$ = 1.0). IFT thus predicts exactly the same predispositions to our mother and father whereas GI predicts a conflict between predispositions (i.e., to a $M_{EXP}$ allele your mother is, genetically speaking, as valuable as yourself, while to a $P_{EXP}$ allele your mother is no more valuable than a stranger [apart from her value as a source of investment in self]). When social behavior has asymmetrical fitness-effects and produces a benefit to, for example, $P_{EXP}$ alleles at a cost to $M_{EXP}$ alleles, $P_{EXP}$ and $M_{EXP}$ alleles are in evolutionary conflict: As a consequence, natural selection of $P_{EXP}$ promotes one behavior and natural selection of $M_{EXP}$ alleles promotes another behavior. (Fig 1, Panel D). But the consequences extend beyond behaviors towards parents. Consider a $P_{EXP}$ allele in a young child. This allele will benefit from motivating behaviors that increase the maternal investment in the child and decrease her investment in the child's siblings. On the contrary, $M_{EXP}$ alleles are best off balancing investment between this particular individual and current, earlier and future maternal siblings. This is because in a promiscuous species like humans, maternal siblings may have *different fathers* while they have the *same mother*[5](Fig 1, Panel B).

Evidence of how $P_{EXP}$ and $M_{EXP}$ alleles act antagonistically has been found in developmental anomalies. For example children with Prader-Willi Syndrome, caused by $M_{EXP}$ on chromosome 15 q11-13 exhibit behaviors that decrease maternal investment, and are characterized by excessive sleeping, low birth-weight, and a diminished appetite directly after birth. But about the time of weaning, when the child no longer relies on breast-feeding, there is a drastic increase in appetite, often resulting in over-eating[11]. Children suffering from Angelman Syndrome, caused by $P_{EXP}$ at the same locus, are characterized by diminished sleep and a smiling disposition[11] that increase attention received from mothers[12]. Thus, $P_{EXP}$ and $M_{EXP}$ might work antagonistically, motivating diametrically different phenotypes within the same individual. When both alleles are expressed (as in healthy individuals), these two phenotypes are in equilibrium.

Studies on mice showed GI in ~1300 loci (most containing genes expressed in the brain)[13,14] with predominant $M_{EXP}$ in cortical regions and predominant $P_{EXP}$ in sub-cortical regions. $P_{EXP}$ and $M_{EXP}$ thus influence function and growth (ontogenetically and evolutionarily) of brain regions differently[15], although the pattern is somewhat more complex[13,14]. A cortical area of particular interest is the medial prefrontal cortex (mPFC) that shows $M_{EXP}$ of the genes Igf2, Dcn, Osbpl5, Copg2, and Ppp1r9a and $P_{EXP}$ of Dio3, Dlk1, Magel2, and Nnat[13,16]. mPFC plays an important role in social cognitive processing, including during early life[17]. As expected, mPFC activity is altered in several imprinted developmental disorders, such as the Prader-Willi syndrome[18–20].Morever, experimental studies using trans-cranial magnetic stimulation (TMS) have shown that this area is involved in regulating human self-enhancement (i.e. the relative value of self over others)[21–23], and neuroimaging studies suggest mPFC is involved in recognition of family members[24,25], altruism and empathy[26,27], child-parent attachment[17], and in the peculiar Capgras syndrome (where the patient believe that familiar persons have been replaced by impostors)[28]. mPFC may thus play an important role in the process of estimating one's own biological value relative to that of close relatives. The anterior cingulate cortex (ACC) is also expected to play an important role. ACC show $M_{EXP}$ of the genes Osbpl5, Copg2, and Ppp1r9a, and $P_{EXP}$ of the Peg1/Mest gene[13]. The dorsal part of ACC is connected with the prefrontal cortex which, in turn, is involved in moderating social behavior[29]. By contrast, the ventral part of the ACC is connected with the hypothalamus that is involved in regulating important nurturing functions, such as aspects of attachment behaviors, hunger, and sleep[30]. ACC activity is also altered in individuals in several imprinted disorders[31,32]. The aforementioned regions have been shown to work together with the temporoparietal junction (TPJ) in integration information of importance to social cognition. In particular, TPJ is involved with self-other distinctions and for considering the perspectives of other's in moral judgments[33,34]. While very little is known with respect to GI patterns in this region, TJP is anatomically and functionally altered in several imprinted disorders[35,36]. While the mPFC is

inferring more enduring dispositions of others and the self, the TJP is more specifically involved in inferring the current goals, intentions, and desires of other people.

Social behaviors with asymmetric fitness-effects are ubiquitous (e.g., all behaviors affecting parents and a large number of behaviors affecting siblings), and I hypothesize that conflict between $P_{EXP}$ and $M_{EXP}$ alleles is an inherent part of decision-making processes regulating social behavior in normal cognitive functioning. I will provide the first experimental tests on this conflict in social behavior in humans. Following earlier evidence[37], conflict will be measured through latency times in decision-making using a paired comparison (PC) paradigm I have developed and successfully used. We will also include electroencephalographic (EEG) measures of conflict. To further explore the neural basis of GI conflict, one experiment involving transcranial magnetic stimulation (TMS) of the brain regions of interest is planned. The research will be conducted in collaboration with internationally renowned researchers providing their expertise on each of the key aspects of the research project. The necessary infrastructure is in place. This innovative research project can be considered high risk. However, the results from two pilot studies (detailed description below) are very promising. The outcome of this research will have the potential to contribute substantially to understanding the role of GI in human social behavior and open up for the counter-intuitive idea that in many situations the self is inherently and genetically divided with regards to the decisions that we make regarding how to best treat others.

## 3 Objectives and Expected Results

My **objective** is to conduct the first investigation of the effects of GI on decision-making regarding kin-directed social behavior. The proposed research will test the **hypotheses** that social decision-making involve internal conflicts between $P_{EXP}$ and $M_{EXP}$ alleles.

Table 1. *Overview of the Main Studies in the Proposed Research Project*

| Study | Focus (Alt.) | Environment | Method | Measures | Sample size | Collaborators |
|---|---|---|---|---|---|---|
| 1 | GI (IFT) | Online Experiment | vPC, | Choices, LT | 2500 | DH, DL, AM |
| 2 | GI (IFT) | LAB Experiment | vPC, | Choices, LT, EEG | 60 | DH, AM, ML |
| 3 | GI (IFT) | LAB Experiment | iPC, | Choices, LT | 90 | DH, DL, AM |
| 4 | GI (IFT) | LAB Experiment | iPC, TMS | Choices, LT | 40 | DH, AM, ML |

*Note:* Alt. = Alternative implementation. GI: Genomic Imprinting IFT = Inclusive-Fitness Theory; vPC = verbal paired comparison paradigm; iPC = image-based paired comparison paradigm; LT = latency time. EEG = Electroencephalography. TMS: Transcranial Magnetic Stimulation. Collaborators' initials (sections 4 & 7) are used.

In **study 1** I will test whether decisions where $P_{EXP}$ and $M_{EXP}$ alleles are in conflict (vs. decisions where $P_{EXP}$ and $M_{EXP}$ alleles are not in conflict) take longer to reach. Latency times in decision-making will be measured in two online experiments in a sample of 2,500 adults (purpose-engineered software is used in the online experiment). Experiment A measures altruistic choices to actual relatives of the participants and experiment B measures inbreeding aversion to actual relatives of the participant. In both experiments, I will use adapted versions of the verbal paired comparison (vPC) paradigm. (Fig 3. Panels A and B). In **study 2**, a subsample of 60 participants who, in study 1 reported having all relatives of interest, will be asked to participate in a lab study. In the lab experiment I will also conduct the same versions (A and B) of the vPC, while recording both latency times and brain activity as measures of internal conflict. In **study 3** I will focus on decision conflict in children (aged 4-8 years) using an image-based paired comparison (iPC) paradigm. Only the altruism iPC will be used. Children are particularly interesting with respect to GI and social decision-making. This is because they still live and interact with family members on a daily basis, which intensifies the benefits of cooperation. The task is similar to that in study 1, but the instructions are presented orally and the children make their choices by

touching an image on a touch screen. (Fig 3. Panel C). In **study 4** I will use trans-cranial magnetic stimulation (TMS) in adults to disrupt activity in mPFC and TPJ, to test whether this is involved in the neural control over fitness-related decision-making. In a repeated-measures design we will disrupt (vs. sham) mPFC and TPJ while asking participants to make decisions in the iPC. We will then compare choice probabilities and latency times to baseline performance.

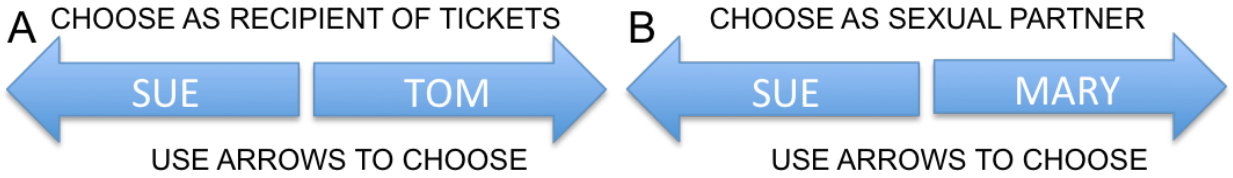



*Fig 3*. **Verbal Paired Comparison. (vPC) Panel A** describes a trial in the altruism vPC. Participants are asked to make a forced choice between two objects (names of the participant's actual relatives, obtained earlier in the study) presented on the screen. The task is to choose to whom of the two relatives they want to give a number of actual lottery tickets. The relatives of interest are selected to represent various degrees of average relatedness and various levels of conflict between $P_{EXP}$ and $M_{EXP}$. The choice and the latency times are recorded as dependent measures, while the degree of relatedness (average, maternal and paternal) between the participant and the relatives presented (e.g., mother vs. paternal half-sister) are automatically coded. The relatedness values can then be used to calculate conflict and predict the actual choice (trial by trial) and the latency times. **Panel B** describes the inbreeding vPC. It is largely similar to the altruism vPC. The only differences concern the task (here the task is to choose the object that is preferred as a sexual partner) and the types of relatives included (only opposite-sex relatives are included; participation to these trials is limited to participants who are not exclusively same-sex interested).


*Fig 4*. **Image-Based Paired Comparison (iPC)**. In the iPC, children are asked to choose one out of two objects (relatives of interest) presented simultaneously on the screen, by touching the with the hand on the face using a tablet computer touch screen. The individual chosen then receives an amount of jellybeans. The jellybeans are visually transferred from the middle of the screen to the face, while latency times are measured from stimulus onset to the touch. The number of jellybeans varies between trials to make it difficult for the children to adopt strategies.

The **scientific impact** of the proposed research is considerable. 1) The proposed research on GI will most likely be the first to show sub-conscious levels of *genetic* conflicts in real-time and 2) that genetic conflict is part of normal cognitive and psychological functioning (as opposed to being part of abnormal functioning). The proposed research has potential for scientific breakthrough as it may come to challenge the widespread consensus that theories need only interpret kin-selection processes at the level of IFT. Due to their novelty and importance of expected results they will be publishable in high-impact journals such as *Science* and *PNAS.* Other publication outlets include dedicated journals such as *Brain, Evolution*, and *Psychological Science.*

Although it is not clear whether it will yield the expected results, the results of pilot studies (described below) are very promising. Given the scientific potential of the project and the promising results from pilot studies, we consider the possible gains to clearly outweigh the risk. I consider as a **critical point for success** that on-line latency times are reliable. To ensure low error variation in response times, I will use a purpose-built program based on HTML5/JavaScript. This type of program shows error below 40ms[38]. It is also important that decisions are non-trivial to the respondents. My earlier studies on inbreeding avoidance have shown that these types of decisions generate the desired level of emotion in participants. I also acknowledge that, although a number of recent studies suggest otherwise, sufficient TMS specificity may be difficult to achieve when targeting the mPFC. To decrease this risk I will collaborate with experienced researchers following rigorous test protocols. If the studies fail to measure maternal-paternal conflict, the procedures allow for an **alternative implementation.**

My prior use of the PC shows that it successfully measures kin-directed social behavior as a function of average relatedness. Because of this, the experiments will provide important and unique data (including brain activity and function) on kin-selection as understood from IFT. The studies will also include new relatives of interest and extend knowledge beyond inbreeding aversion to also include altruism. Our unique access to large and representative samples allows for exceptional statistical power, very exact estimates of effect sizes, and highly generalizable results. To facilitate new research in the area I will publish data on public, generalist repositories (figshare.com or datadryad.com). This will be done after carefully making sure that the anonymity of the participants is not compromised. I will opt for an **open-access publication policy**. A preference will be given for open access journals. If publishing in traditional journals, articles will be made publicly available on the department homepage after the embargo has expired. I will also publish comments on each study on my personal homepage (www.jantfolk.com) and make published articles available through Research Gate (www.researchgate.net/jantfolk). Press releases will also be prepared through the university's Press Office and through the Academy of Finland.

## 4 Research Methods and Materials, Support from Research Environment

In **Studies 1** and **2** I will measure both altruism and inbreeding avoidance in adults. Two versions of the vPC have been created. In the altruism vPC, participants are asked to allocate a number of actual lottery tickets (for a 500 € gift-card) to either one of two objects presented on the screen. In the inbreeding vPC, the task is to choose one of the two objects as a relatively more attractive sexual partner. Each object is a name of an actual relative (out of a fixed list of possible relatives of interest) or of the participant him/herself. Relatives of both sexes and the participant themselves appear as objects in the altruism PC but only relatives of the opposite-sex can appear as objects in the inbreeding PC (See Table 2).

Table 2 *Degrees of Relatedness and Maternal-Paternal Conflict (Asymmetry) for Relatives Included in the PCs*

| Relatives of Interest | Altruism | Inbreeding[a] | Relatedness | | | Asymmetry |
|---|---|---|---|---|---|---|
| | | | $r_{AVG}$ | $r_M$ | $r_P$ | |
| Participant | 1,2,3,4 | | 1.0 | 1.0 | 1.0 | - |
| Mother | 1,2,3,4 | 1,2 | 0.5 | 1.0 | 0.0 | 1 |
| Father | 1,2,3,4 | | 0.5 | 0.0 | 1.0 | -1 |
| Sister* | 1,2,3,4 | 1,2 | 0.5 | 0.5 | 0.5 | - |
| Brother* | 1,2,3,4 | | 0.5 | 0.5 | 0.5 | - |
| Maternal Half-Sister* | 1,2,3,4 | 1,2 | 0.25 | 0.5 | 0.0 | 0.5 |
| Maternal Half-Brother* | 1,2,3,4 | | 0.25 | 0.5 | 0.0 | 0.5 |
| Paternal Half-Sister* | 1,2,3,4 | 1,2 | 0.25 | 0.0 | 0.5 | -0.5 |
| Paternal Half-Brother* | 1,2,3,4 | | 0.25 | 0.0 | 0.5 | -0.5 |
| Maternal Female Cousin | 1,2 | 1,2 | 0.125 | 0.25 | 0.0 | 0.25 |
| Maternal Male Cousin | 1,2 | | 0.125 | 0.25 | 0.0 | 0.25 |
| Paternal Female Cousin | 1,2 | 1,2 | 0.125 | 0.0 | 0.25 | -0.25 |
| Paternal Male Cousin | 1,2 | | 0.125 | 0.0 | 0.25 | -0.25 |
| Daughter | 1,2 | 1,2 | 0.5 | 0.5 | 0.5 | - |
| Son | 1,2 | | 0.5 | 0.5 | 0.5 | - |

*Note:* For simplicity, the table considers only male participants. Because nothing is known about inbreeding avoidance in homosexual individuals, the study focuses on heterosexual participants. Therefore, the inbreeding paradigm only includes opposite-sex relatives. [a]Only relatives older than 16 years of age are included in the inbreeding PC. This choice is made to conform to the Finnish Legal Definition of Sexual Abuse. *Two relatives of this category can be included, given that the participant has two or more relatives belonging to these categories and that the maximum number of possible comparisons has not yet been met. Asymmetry is counted by subtracting paternal relatedness from maternal relatedness. Positive values indicate higher maternal relatedness.

Because the number of relatives of interest is relatively high (altruism PC = 21; inbreeding PC = 10), this yields a high number of possible comparisons (altruism PC = 210 [(21x20)/2]; inbreeding PC = 45 [(10x9)/2]). To not exhaust participants, a limit will be set to 9 relatives. A preference order will be predefined, such that highly interesting (i.e., categories with high levels of conflict) categories (mothers, fathers, older siblings, younger siblings, maternal and paternal half-siblings) are prioritized and a

limit is set on the total number of possible comparisons a participant can receive. A limit of 9 relatives gives max 36 comparisons ([9x8]/2). For each participant, each comparison will appear 4 times. This will allow us to estimate the reliability of this procedure. A participant who has 9 or more relatives of interest will thus complete a maximum of 144 trials (36 x 4). Because trials take ~3.5s to complete (including ISI, fixation time, and response latencies), each PC can be completed in less than 8 minutes. To limit tiredness effects, trials will be separated into two blocks. Because the aggregated data file is large (altruism PC = 210 cells; inbreeding PC = 45 cells), and the number of data points obtained from each participant is limited (≤ 144), we need a large amount of participants to secure sufficient observations for each cell. Based on an earlier study, we estimate that participants will provide on average 88 data points. This gives more than 200,000 observations. With this number of observations, we will secure sufficient number (>250) of observations also in the least prioritized cells. Considering only odds of 1:1.5 (corresponding to half of the minimum relative distance in relatedness between two individuals) as interesting, this sample size would give us sufficient statistical power (>80%).

Based on the responses a participant gives in study 1, 60 participants will receive an invitation to participate in **Study 2.** Each participant who, in study 1 reported all relatives of interest for study 2, will be asked to sign up for the lab experiment. The first 30 male and the first 30 female participants who sign up will be selected. The number of possible trials per comparisons will be increased (7 trials per comparison, totaling 288 trials per person in the altruism PC and 12 trials per comparison, totaling 252 trials per person in the inbreeding PC). This increase is done in order to achieve a high signal-to-noise ratio in analyses of brain activity. Again, trials will be separated into two blocks for both PCs. The same data will be recorded in study 2 as in study 1, but with the addition of also recording **EEG-data**. **EEG-data** will be collected with the NeuroOne system (Mega Electronics Ltd, Kuopio, Finland) at the Center for Cognitive Neuroscience (University of Turku) using the international 10/20 electrode-placement system. The EEG analysis will focus especially on the frontocentral N2 component (N2b) that is generated in the ACC and related to cognitive control and conflict resolution[34,35]. It is a negative event-related potential wave peaking around 200-350ms after stimulus onset. I will quantify the N2b as the base-to-peak voltage difference between the most negative peak within a window of 150ms to 500ms and the preceding positive peak. The magnitude of this waveform is expected to be associated with genetic conflict between the two objects presented in a trial.

In studies 1 and 2 we expect that decisions are i) predictable from IFT, that ii) asymmetric (vs. symmetric) decisions are harder to reach, and iii) that this hesitancy is also reflected in neural activity. The studies test whether GI is involved in kin-selection processes in humans.

The iPC in **Study 3** uses photographs of the relatives of interest instead of the names. The necessary photographs will be obtained through the children's parents and will be standardized (size, brightness, and by removing everything else but the face of the child) using Adobe Photoshop CC. We will use a 12.9" iPad Pro with a touch sensitive screen for presentation. We will select a lower number of comparisons, focusing on the parents, siblings, and half-siblings only. This gives us an upper limit of 14 relatives of interest. (Table 2). The number of relatives for each participant is limited to 6. This gives us 15 comparisons [(6x5)/2], all repeated 4 times. The reason for this limitation is to not exhaust the children and decrease the amount of preparation needed in handling the photographs. We will sample 30 4-years old, 30 6-years old, and 30 8-years old children. This will allow us to gain knowledge about the developmental aspects in kin-selection. An ongoing pilot study has shown that children as young as 4 years understand the task at hand.

In **Study 4** we will use a repeated measures design dispersed over three sessions. 48 participants will make decisions using both the altruism iPC. The iPC is similar to that of study 3 and contains 60 trials. First, individual baselines for choice probabilities and latency times will be collected. After this participants will randomly be assigned to either of two trial sequences (A = Sham, B = TMS or A = Sham, B = TMS). Both the sham condition and the TMS condition targets

three locations (mPFC, rTPJ, and lTPJ) and the order of these locations is counterbalanced within participants and conditions. After the experiment is completed, participants will be asked to conduct a facial recognition task. In this they will see each of the images again and provide the name of the depicted individuals (Both sham and TMS). This is done to test whether possible effects are due to effects on facial recognition. We expect that disrupting the interplay between mPFC and TPJ will increase the relative value of self over others, and lead to a self-biased strategy in the altruistic decision-making (i.e. choice probabilities will no longer be predicted by IFT). Moreover, we expect that disrupting cortical control functions will lead to a father-biased strategy in decision-making (i.e., paternal relatives will be given more value than maternal relatives). By including both mPFC and TPJ areas (both vs. sham), we will be able to dissociate she specific contribution of these areas. The results of this study will contribute knowledge on the neural basis of kin-selection.

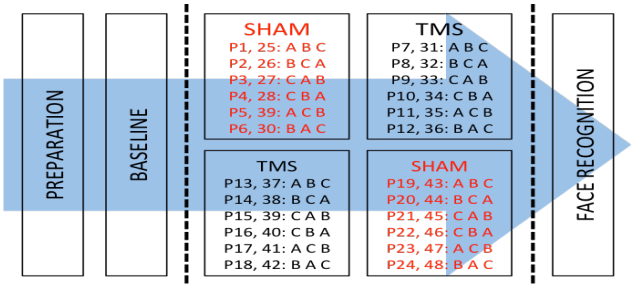



*Fig 4.* After baseline assessment (session 1) participants are assigned to either of two condition sequences (sham or TMS) dispersed over two independent sessions (2 & 3) In both conditions three locations (A = mPFC, B = RTPJ, C = LTPJ) will be targeted. The order of these locations is counterbalanced within participants. After completion, participants conduct a facial recognition task. Single biphasic TMS pulses will be administered with a Nexstim Ltd. eXimia$^{TM}$ stimulator and a Nexstim biphasic 70 mm figure-of-eight coil while reducing TMS-induced noise and fixing the head position using a chin rest. Before stimulation, a high-resolution T1-weighted anatomical MRI-image of the brain will be acquired from participants. Impulses will be directed to targeted areas using the Navigated Brain Stimulation system that registers the relationship between the coil and the brain with a spatial resolution of 2 mm. P1-48 = participant numbers.

In all studies **choices** are binary and can be analyzed both on a trial-by-trial level and aggregated (within a comparison type and across comparison types). Choice probabilities are counted as the number of times one object is chosen over another, divided by the number of times they have been compared (x/[x+y]). Note, however, that GI does not predict that choice probabilities differ from those predicted by IFT. Rather, GI predicts that decisions should become increasingly hard to reach (delaying latency times) when there is a high conflict between $P_{EXP}$ and $M_{EXP}$ genes. **Latency** times, considered as a reliable measure of conflict in cognitive tasks[37], will be measured as the time between stimulus onset and the decision being made (key press). When using a keyboard, latency time measures contain small but not irrelevant error-variation due to the electronics in the particular hardware. To record response latencies in the lab (Studies 2 and 4), I will therefore use a 36 Channel Elite Black Box Tool Kit that allows subtracting a consistent measurement error increasing accuracy in reaction-time measurement.

Important **covariates** will also be measured in all studies. For kin-selection—whether we consider IFT or GI—to operate, kin needs to be identified and categorized. Only women can, due to the nature of gestation, be certain about their biological relatedness to their children. *All* other human family relations are probabilistic in nature. To identify kin humans rely on various types of ecological cues[39–41]. For example, older children who witness their mother caring for a younger children use this particular cue to identify a sibling or a maternal half-sibling. The younger child, to whom this cue is unavailable, uses shared co-residence with the older child as a cue of siblingship[39]. Differential selection of maternally and paternally inherited alleles also necessitates a capacity to separate between maternal and paternal relatives. Within the animal kingdom, parent-specific kinship cues are expressed in offspring and modulate social behavior[42].

The presence/absence of these cues affect the psychologically estimated degree of relatedness. My earlier studies have shown that by measuring these cues and the psychologically estimated degree of relatedness, kin-directed decisions can be predicted to even a higher extent than when using only the biological estimate of relatedness. Because of this, measures of all the known cues for categorizing individuals into each of the aforementioned categories will be used and included in the data analyses. Also experimentally induced covariates will be considered. These include, for example, the temporal location of a trial in a series of trials and the length of the names presented on the screen. These will be automatically recorded.

**Pilot Studies.** In **Pilot 1,** 356 participants made decisions about how to distribute resources between family members using an adapted version of the vPC**.** In this on-line version of the PC the task was to allocate a number of lottery tickets to either one of two objects (names of actual relatives) presented on the screen. I found that 1) the magnitude difference in the inclusive fitness-gain between two objects presented in a trial correctly predicted 60% of the actual choices made. To test if the conflict between $P_{EXP}$ and $M_{EXP}$ alleles increases reaction times trials where one of the two scenarios was preferable from a maternal interest point-of-view whereas the other was preferable from a paternal interest point-of-view (high conflict) yielded, on average, 720.0ms ($SD$ = 489.9, $p < .05$) longer reaction times than in trials where the same scenario was preferable from both points-of-view (low conflict). This pilot study had some limitations. Firstly, this vPC did not include the parents of participants (that yield strong conflicts) but only horizontal relatives. Secondly, the sample was not specifically targeting individuals with all relatives of interest, which increased the amount of participants providing non-informative trials, which, in turn, decreased the signal-to-noise ratio. Therefore we also conducted a pilot in the lab.

In **Pilot 2** we used both the altruism vPC and the inbreeding vPC. Participants were chosen so that they had at least one half-sibling, a mother, and a father. To be included in the inbreeding PC it was also necessary to have an opposite-sex half sibling. Four of these participants participated successfully in the altruism PC and three participants participated in the inbreeding PC Participants made between 144 and 240 decisions each in the altruism PC. In the inbreeding PC, each participant made 144 decisions. First we investigated whether choice probabilities could be predicted by IFT. We found that in both the altruism PC ($B$ = 1.38, $SE$ = .20, $p < .001$, $n$ = 528; 66.1%) and the inbreeding PC ($B$ = -6.57, $SE$ = .74, $p < .001$, $n$ = 216; 83.8%) a high proportion of choices were correctly predicted (participants were more likely to give lottery tickets to an individual they were relatively highly related to, and participants were less likely to prefer having sex with an individual they were relatively closely related to). We then investigated the effects of conflict between $P_{EXP}$ and $M_{EXP}$ on latency times. We first compared all trials with no conflict between $P_{EXP}$ and $M_{EXP}$ with all trials that implied a conflict. In line with our expectation, we found longer latency times in trials with a conflict compared to trials with no conflict in both PCs ($M_{DIFFERENCE}$ = 575.69ms, $SE$ = 183.93, $p < 001$ in the altruism PC and $M_{DIFFERENCE}$ = 320.28ms, $SE$ = 196.73, $p < .05$ in the inbreeding PC). As expected, although there was a mean difference of in latency times, the choice probabilities were not affected by conflict ($p > 0.5$ in both PC), suggesting that the time it took to reach a decision was prolonged by conflict but that the outcome was not. There was, however, a slight correlation between the average relatedness and levels of conflict across the trials. To suppress the possible effect of difference in average relatedness we next only selected trials were the average relatedness was equal to both objects on the screen. Unfortunately, this was possible only in the altruism PC because no trials where both objects were equal in terms of IFT had appeared in the inbreeding PC. Again, we found that a conflict between $P_{EXP}$ and $M_{EXP}$ (vs. no conflict) was associated with an increase also in latency times ($M_{DIFFERENCE}$ = 701.04ms, $SE$ = 203.74, $p < 001$) in this subset of trials in the altruism PC. After this, we explored the ERP responses. We found of an altered N2 response in the right hemisphere due to the relatedness to the stimuli on screen, suggesting that the difference in average relatedness between stimuli and the conflict (vs. no conflict) was associated with the NW response. There was not enough statistical power to

perform meaningful statistical tests on the ERP responses from these three subjects. (Fig 4).

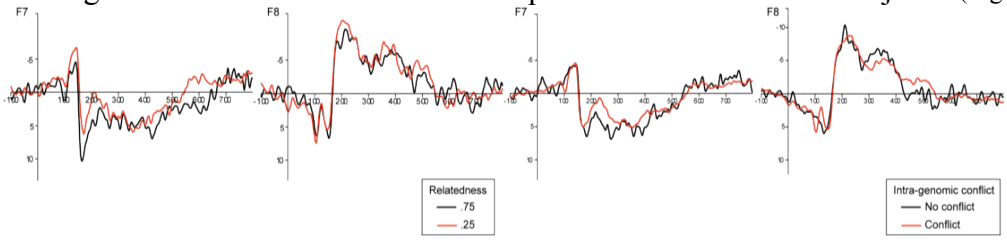



*Fig 4*. ERP responses for trials with different relatedness values between objects (Left) and different levels of conflict (Right). The N2 response around 200ms, particularly in the right hemisphere was influenced by the manipulation in both contrasts. Because the number of participants was low, no statistical inferences can be made.

*Sample and Compensation to Participants*

The Finnish Population Registry Centre holds information on all Finns and their family structures. This affords the unique opportunity to obtain representative but targeted samples of the necessary populations (e.g., individuals with both maternal and paternal half-siblings) for **Studies 1**, **2** and **4**. For **Study 3** participants will be recruited by sending invitation letters to all primary schools in southern Finland. Participants in **Studies 1**, **2** and **3** participate in a lottery of a 500 € gift-card. In studies 2, 3, and 4 participants receive 50 Euros for their participation. In studies 1, 2 and 4 participants will be informed that each of the relatives included will also obtain the allocated number of lottery tickets. This is for participation of an additional gift card worth 500 Euros. In **Study 3**, participants and their family will receive tickets to a nearby theme park. They (and their relatives) will also receive jellybeans in accordance with the child's choices.

*Research Materials and Research Environment*

Delosis Ltd. (London, UK, www.delosis.com) has produced a task that obtains decisions and reaction times and can be used both online and in the lab (example).

A weekly seminar will be held with a focus on the proposed research at the faculty. In this seminar I will make use of three of the central research areas at IPL at ÅAU: behavioral genetics, cognitive neuroscience, and evolutionary psychology. The behavioral genetics and the evolutionary psychology group are led by Prof. **Pekka Santtila** and have published extensively on genetic predispositions in behavioral and psychological phenotypes and how social decisions relate to inclusive fitness. Prof. **Matti Laine** leads the cognitive neuroscience group. He has vast experience in cognitive neuroscience applying different of methods in his research, including experimental cognitive measures and functional brain imaging methods such as EEG. His research group has published in high-impact journals such as *Science*, *Brain*, and *Journal of Neuroscience*. I will also have access to the EEG and TMS equipment at the Centre for Cognitive Neuroscience at Turku University, where **PhD. Myong Kwon** (senior researcher), who has been using EEG for more than ten years, and **PhD. Henry Railo**, who has expertise in TMS methodology (including combining TMS and EEG), will assist me in technical aspects of the experiments, as well as data acquisition and data analysis.

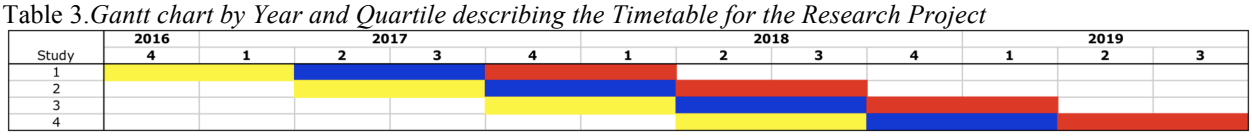
## 5 Ethical Issues

Studies 1 and 2 involve only adult volunteers. Participants in all studies will give their informed consent before participation. No deception is necessary and information that could compromise confidentiality (e.g., names of relatives) will not be saved together with participant's responses. The Ethical Review Board at the IPL at ÅAU has approved **studies 1 and 2** (including collecting EEG data). **Study 3** involves children. This means that their legal guardian(s) will give their informed consent. No deception will be necessary and the images necessary will only be stored in a encrypted and password-protected file on non-public departmental computer. Only the PI will have access to the data. Only anonymous and aggregated data will be published as

supplementary material for individual studies. Images and files used in their preparation will be deleted after the experiment. The ÅAU Ethical Review Board has approved a pilot study for **Study 3**. A separate application for **Study 4** will be made. Henry Railo has ethical board approval to employ single-pulse TMS to study cognition (Ethical Board of the South-West Finland Hospital District). With proper screening of participants, stimulation parameters, and the use of hearing protection TMS can be considered a low-risk method. We will use single-pulse TMS which has an extremely low risk of inducing seizures. Only anonymous and aggregated data will be published as supplementary material for individual studies.

## 6 Implementation: Schedule, Budget, and Distribution of Work

Table 3.*Gantt chart by Year and Quartile describing the Timetable for the Research Project*




*Note:* Yellow = Planning; Blue = Data Collection, Red = Data Analyses and Preparation of Manuscripts; AAU = Åbo Akademi University, UM = University of Miami, UZ = University of Zurich

Table 4. *Budget of the Proposed Research Project*

| Source | Extent/Use | Costs |
|---|---|---|
| Postdoc salary | Principal investigator for 36 moths | 130640 |
| Materials | (Software, data collection costs, and brain activity-analyses) | 24000 |
| Services | (Data analyses, and compensation to participants) | 18000 |
| Travel expenses | (Meetings of the group, 1/year) | 19000 |
| Mobility | 6+2+3 months x 1500,- | 16500 |
| Overhead | | 223446 |
| TOTAL | | **4317586** |

## 7 Research Team and Collaboration

To ensure expert knowledge on crucial areas in this research proposal, it will be conducted in a network of world-leading experts. All experts have reviewed this grant application and agreed on the network structure, mobility, and the distribution of work. **David Haig** (professor, Department of Organismic and Evolutionary Biology at Harvard University, US) has developed the kinship theory of GI and is involved in several research projects on the effects of GI. His outstanding publication record includes several influential publications in high impact journals such as *Science,* and *Proceedings of the National Academy of Science.* I will visit DH at his department prior to the start of the proposed research. **Debra Lieberman** (professor, Department of Psychology at University of Miami, US) is a renowned expert on kin-identification systems and their role in modulating social behavior. Her publications have been published in journals such as *Nature* and *Proceedings of the Royal Society B: Biological Sciences.* I have previously worked together with DL in a research project financed by the Academy of Finland (no. 260298), in which I was employed as a PhD-student. **Andreas Mokros** (senior researcher, University Hospital of Psychiatry Zurich, CH) is an expert on decision-making paradigms as measures of real-life preferences. He also has specialized competence in analyzing paired-comparison data. I have previously worked together with AM on two other another research projects funded by the Academy of Finland (no. 121232 and 260298) and the *MiKADO* project funded by the German Family Ministry.

## 8 Researcher Training

The proposed research expands on my prior research within evolutionary psychology in general and kin selection in particular. The collaborations will increase my competence within several fields. A subsequent application for a **doctoral student** (N.N) will be submitted. N.N. will focus his/her thesis on the brain correlates of GI. I will supervise N.N together with Prof. Matti Laine.

Each data collection will also be conducted in collaboration with 1-2 master students. In line with the AAU profile, I will strive for a balance between genders in all recruitment.

## 9 Mobility Plan

In 2017 I will stay 6 months with Prof. Lieberman at the University of Miami, US to plan and develop measures of kin recognition. I will also stay at Harvard University during 2016. During this time period, further tests of the effect of GI on social behavior in humans will be planned. I will also visit Dr. Mokros at his department at the University Hospital of Psychiatry Zurich for 2 x 1 month to conduct analyses on the GI under his supervision.

## 10 Key Literature of the Principal Investigator and the Members of Research Group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