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Functional Linear Models Extensions Uncover Pleiotropic Effects of Chronic Pain Phenotypes

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Background & Motivation

Growing scientific evidence suggests that intricate interactions of genetic risk factors with environmental exposures play a major role in the development of chronic pain conditions. In studies of relative contribution of an individual's genetic composition to the perception of pain, the general characteristics of pain sensitivity are typically measured by a wide range of different, yet possibly etiologically related pain phenotypes.

Testing each of these pain-perception traits individually is subject to problems of multiple testing and low statistical power. Furthermore, pain-related traits may share common etiology and comprise binary, categorical, and quantitative measurements.

In the current study, we propose a novel statistical approach for simultaneous testing of multiple correlated phenotypes, including quantitative binary, categorical or a combination thereof, with the flexibility of adjusting for other covariates, to explore whether variation within the coding sequence of the gene encoding the P2X7 receptor (*P2X7R*) affects chronic pain sensitivity in humans.

Materials

P2RX7 encodes an ionotropic purinergic receptor central to the pro-inflammatory response; its genetic variation has been recently connected to chronic pain. Previously published results suggest an association between *P2RX7* genotype and the amount of allodynia and chronic pain in mice and in humans (Sorge et al. (2012)).

Fibromyalgia and chronic pelvic pain are central sensitivity syndromes and may share genetic etiology. To test whether chronic pelvis pain sensitivity and fibromyalgia are associated with *P2RX7*, we obtained information over 53 single nucleotide polymorphisms (SNPs) with minor allele frequency (MAF) ≥ 0.05 in a cohort of 2,703 subjects from the OPPERA (Orofacial Pain: Prospective Evaluation and Risk Assessment) project. Covariates included gender, OPPERA site, case/control status of temporomandibular disorder (TMD), and the first three principal component analysis vectors accounting for variability due to ancestry.

	Male ($n = 1,034$)	Female ($n = 1,669$)
Percent TMD Cases	19.8%	38.6%
Percent Chronic Pelvic Pain	0.8%	2.2%
Percent Fibromyalgia	0.9%	3.1%
OPPERA Site		
Florida	25.5%	26.8%
Maryland	27.7%	20.1%
North Carolina	23.0%	31.1%
New York	23.7%	21.9%

Table 1: Distribution of pain sensitivity and baseline enrollment status, separately for male and female, based on data from the OPPERA study.

Methods

The 'Flipping' Algorithm

SNP_1	SNP_2	Flip?
2	0	✔
2	0	✔
0	2	✔
0	1	✖
0	1	✖
0	1	✖
1	1	✖
	Sum=3	

Relabel SNP_2 ⇒

SNP_1	SNP_2	Flip?
2	2	✖
2	2	✖
0	0	✖
0	1	✖
0	1	✖
0	1	✖
1	1	✖
	Sum=0	

Table 2: Sample genotype information over seven subjects and two SNPs. *Left panel* illustrates original data and highlights subjects for which a flip in genotype coding at SNP_2 position is possible. *Right panel* illustrates 'flipped' data at the SNP_2 position.

Prior to the analysis, we implemented the 'flipping' algorithm that minimizes the number of 0-2 (or 2-0) patterns in genetic data (with SNP coding based on minor allele frequency). The result of association analyses that do not make a priori assumptions about the direction and the magnitude of effects should not be affected by 'flipping', but the power of our statistical analysis is enhanced (Vsevolozhskaya et al. (2014)).

Genetic Data as Smooth Curves – 'Genotypic' Functions

After the 'flipping' algorithm, for each subject, we obtain a 'genotypic function' – a nonparametric function fitted with a basis expansion method. Thus, the genetic data is no longer of a discrete nature, such as would be the case for allele frequencies, but rather a single nonparametric genotypic function, $G(t)$, of a continuous nature.

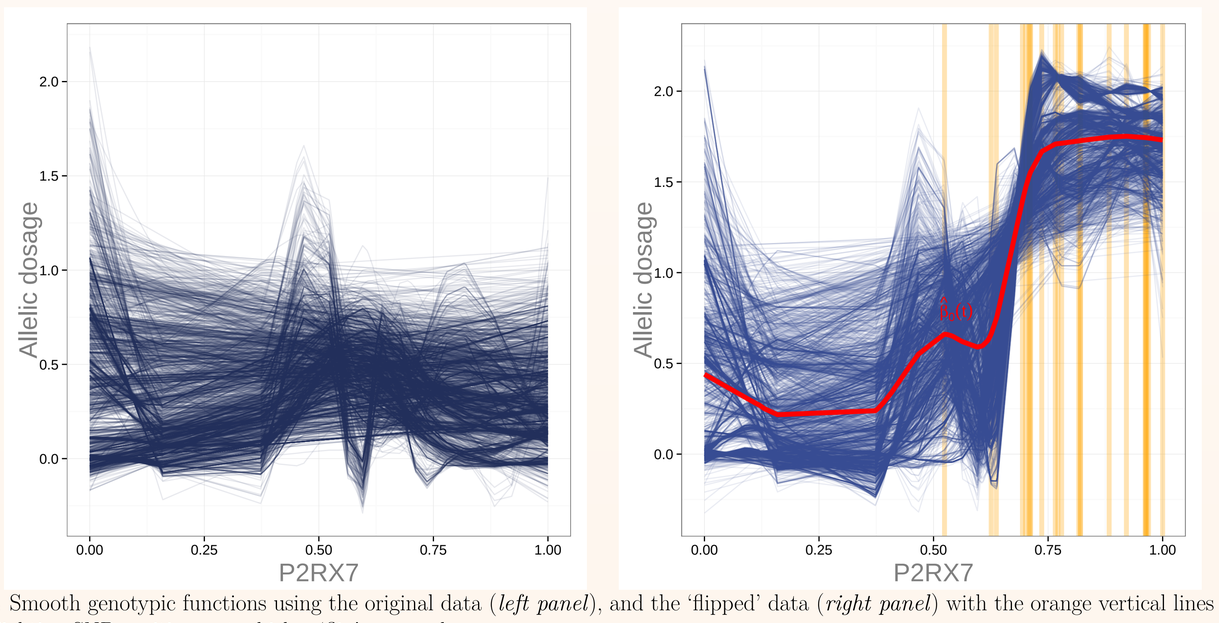

Figure 1: Smooth genotypic functions using the original data (*left panel*), and the 'flipped' data (*right panel*) with the orange vertical lines highlighting SNP positions, at which a 'flip' occurred.

The Model

Unlike traditional statistical models that treat a disease phenotype as an outcome (i.e., on the left-hand side of the equation), our model puts all traits on the right-hand side, including disease status, environmental exposures, and covariates. The response variable is a genotypic function for each individual.

$$\hat{G}_i(t) = \hat{\beta}_0 + \underbrace{\hat{\beta}_1(t)X_1 + \cdots + \hat{\beta}_p(t)X_p}_{\text{pleitropic pain phenotypes}} + \underbrace{\hat{\alpha}_1(t)C_1 + \cdots + \hat{\alpha}_m(t)C_m}_{\text{covariates}}$$

Testing for effects or for validity of regression adjustments are preserved under the reversal due to the invariance of the partial correlations.

Results

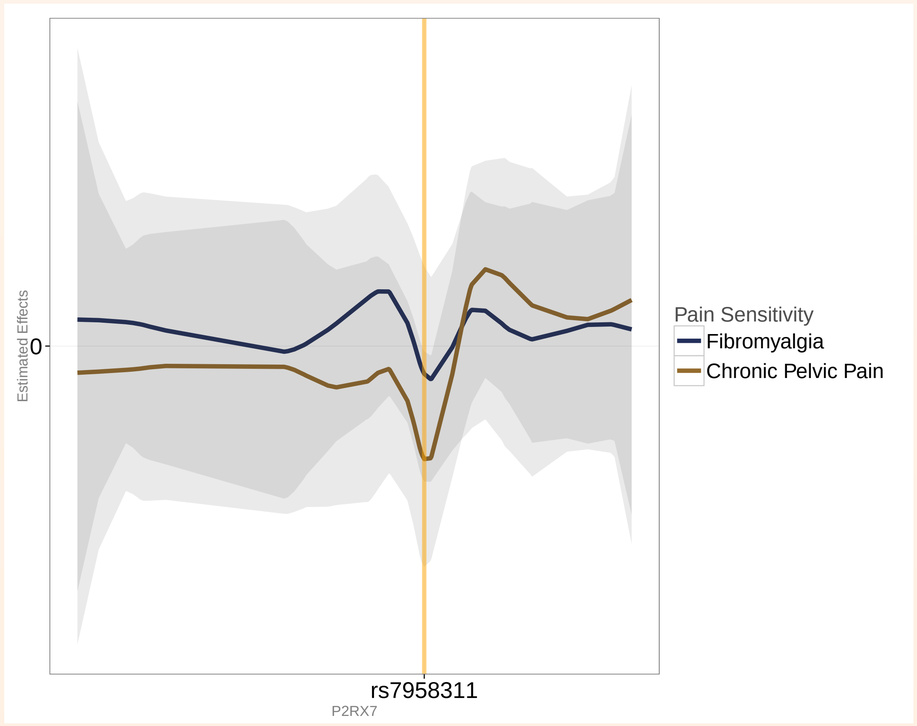

Figure 2: Estimated fibromyalgia (blue) and chronic pelvic pain (brown) effects with the corresponding 90% CIs.

We tested for an association between 53 SNP variations in *P2RX7* that were identified among 2,703 participants in the OPPERA study and two pleiotropic chronic pain conditions – chronic pelvis pain and fibromyalgia. Additional covariates included gender, OPPERA site, TMD case/control status, and the first three principal components. The *P*-value for association between two chronic pain conditions and *P2RX7* was 0.197. Next, we proceeded to investigating the functional effects of the two chronic pain phenotypes. Functional effects, $\hat{\beta}(t)$'s, allow to unveil a more nuanced blueprint of how phenotype effects may vary within a genetic region. Specifically, $\hat{\beta}_0(t)$ – a functional intercept, – estimates smooth baseline allelic dosage over multiple SNPs in the population (highlighted in Fig.1). Fig. 2 shows estimated effects of the chronic pelvis pain and fibromyalgia, i.e., estimated deviations from the baseline allelic dosage, with the 90% point-wise confidence intervals. The area around rs7958311 represents the highest hit with the strongest association with chronic pelvic pain. The direction of the effect suggests that carries of rs7958311 may have a higher risk of chronic pelvic pain (note: rs7958311 minor allele frequency coding was 'flipped'). rs7958311 is a part of the haploblock, containing gain-of-function variants rs208294, rs1718119, and rs3751143 that may comprise a pain protective haplotype.