studies might benefit from continuous, rather than ordinal, risk response designs.

Self-reporting error is another limitation of our study. This issue is a well-known challenge in survey design. We limited our medical usage outcomes to survey questions asking about discrete events that clearly linked action to PGT results to minimize vague interpretations. But self-reporting error cannot be eliminated under this research design. Furthermore, the 6-month risk perception changes and medical usage choices are only a snapshot of an individual's beliefs and behavior. Longer-term tracking and longer follow-up surveys would be needed to understand how these perceptions and actions evolve over time.

Finally, the study can measure only the increases in healthcare usage as a result of increased risk perceptions and does not provide conclusions about the overall health value of PGT. Decreased risk or good news PGT results might give a consumer false reassurance and lead to a reduction in healthcare usage or health-enhancing behaviors. An ideal study design might include an individual's entire medical history as well as measures of health behaviors both before and after PGT. However, the time horizon and survey limitations of the PGen Study did not allow this. Whereas the study provides a step toward better empirical understanding of the psychological and behavioral impact of PGT, the data do not allow us to measure the long-term health benefits (or costs) of PGT. We hope this study will encourage future attempts for linking information interventions with medical records and long-term behavioral tracking, as well as qualitative data on risk perception.

A frequently cited concern regarding the regulation of DTC genomics is a lack of understanding about how individuals respond to the information presented in these tests. Our results provide early evidence of how customers adjust their perceptions and engage with their health providers as a result of different types of PGT results. Though we found good news–bad news asymmetry in risk perception changes, these changes appeared to be moderate and congruent with test results. Furthermore, extreme perception changes drove much of the follow-up medical appointments and procedures. Taken together, our results suggest that DTC consumers learn from their PGT results and update their beliefs, but they primarily seek additional medical actions in response to large and unexpected risks.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper (doi:10.1038/nbt.3661).

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AUTHOR CONTRIBUTIONS
J.L.K. formulated the research question, designed and performed the analyses, prepared the figures and wrote the manuscript. F.M. helped with the research design, analyses, and manuscript writing. R.C.G. and J.S.R. designed and implemented the surveys, in their roles as the primary investigators of the Impact of Personal Genomics (PGen) study, and advised on the preparation of the figures and the manuscript. All authors edited and approved the manuscript.

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Containment of transgenic trees by suppression of *LEAFY*

To the Editor:

Field studies and commercial use of genetically engineered (GE) trees have been limited, in large part owing to concerns over transgene flow into wild or feral tree populations[1–4]. Unlike other crops, trees are long-lived, weakly domesticated and their propagules can spread over several kilometers[5]. Although male sterility has been engineered in pine, poplar, and eucalyptus trees grown under field conditions by expression of the barnase RNase gene in anther tapetal cells[6,7], barnase can reduce rates of genetic transformation and vegetative growth[6]. Furthermore, barnase expression may not be fully stable[8]. Bisexual sterility would allay concerns over seed dispersal, could be used to control invasive exotic trees, and might increase wood production[9]. We report the use of RNA interference (RNAi) to suppress expression of the single-copy *LEAFY* (*LFY*) gene to produce sterility in poplar.

RNAi has been used to reduce gene expression in many plant species[10,11], and the reduction in gene expression that RNAi confers is highly stable in trees under field conditions[12]. *LFY* is required for the early stages of male and female floral organ formation in plants, and encodes a transcription factor that promotes floral meristem identity[13,14]. In *Arabidopsis thaliana*, loss of *LFY* function results in the formation of vegetative structures instead of floral meristems, whereas reduction of *LFY* expression decreases floral abundance and results in partial conversion of floral organs to leaf-like structures[13,14]. We selected *LFY*

for the production of sterile poplar trees because it is highly conserved, usually present as a single copy, and is expressed at basal levels in vegetative tissues[15].

We produced an RNAi construct based on the *Populus trichocarpa* (*P. trichocarpa*) *LFY* sequence (*PtLFY*, **Supplementary Methods** and **Supplementary Fig. 1**). We transformed a female clone of white poplar, *Populus alba* (*P. alba*) genotype 6K10 with our RNAi–*PtLFY* construct. This clone was chosen because it begins to flower 2 years after planting under growth-promoting conditions, which is far earlier than most other tree species or varieties of poplar (which usually begin to flower at ~5 years). Sequence analysis of the *LFY* cDNAs from *P. trichocarpa* and *P. alba* showed that they were 98.15% identical across their coding sequences, and 98.62% identical across the region used for the RNAi inverted repeat (**Supplementary Fig. 2**). We isolated 15 independent RNAi–*PtLFY* transgenic events and planted four ramets (genetically identical trees) per event (**Supplementary Table 1**), in the field, together with 24 non-transgenic 6K10 control trees. These trees were planted near to Corvallis, Oregon, in 2011, as part of a larger field trial (USDA-APHIS-BRS-approved permit 10-260-102r-a1; current permit is 13-330-102r). The experiment included 23 constructs and three separate poplar clones (genotypes). Tree survival was scored yearly for all trees. The plantation experienced an ice storm in February 2014 and unusually hot and dry conditions during the summers of 2014 and 2015. All 24 non-transgenic 6K10 control trees and 59 of the 60 RNAi–*PtLFY* trees were alive in 2015 (**Supplementary Table 1**).

In January 2014, we screened all 6K10 trees including all 24 non-transgenic control and all 59 RNAi–*PtLFY* trees for the presence of floral buds, which can be distinguished from vegetative buds by their size and shape[16]. We found that 14 of the 15 RNAi–*PtLFY* events had floral buds in 2014, with 0–100% flowering within events (**Supplementary Table 2**). As poplar buds at this stage are ecodormant (dormant due to temperature, not internal factors), we collected twigs with floral buds to force them to flush under warm temperatures in the laboratory. This provided a first view of floral morphology. Although 12/14 tested RNAi–*PtLFY* events in 2014 had floral buds and flowers similar to those of the control trees, 2 of the 14 independent events (17 and 139-1) had smaller floral buds than the controls. These small floral buds flushed 4 days later than other events and had small catkins (**Fig. 1**). Both events 17 and 139-1 also had small catkins that opened late, in the field, failed to enlarge, and did not produce the cotton-like material to which poplar seeds are normally attached. In 2014, the small catkins fully emerged in April, and control catkins opened in early March (**Fig. 1**). Floral production and phenotypes of all planted events were re-analyzed in early 2015 (between January and early April). We used the 2014 observations to develop a scoring system for 2015 floral bud opening and catkin development (**Supplementary Fig. 3**). All 15 RNAi–*PtLFY* events flowered in 2015, with events 17 and 139-1 both showing delayed floral opening again compared with all the controls and the other 13 RNAi–*PtLFY* events (**Fig. 1** and **Supplementary Fig. 4**). Both of these sterile events (17 and 139-1) had a 100% rate of flowering in 2015 (**Supplementary Table 3**). Floral phenotypes were similar for all events tested in the 2014 and 2015 growing seasons (**Supplementary Tables 2** and **3** and **Supplementary Fig. 4**). Similar results were also obtained

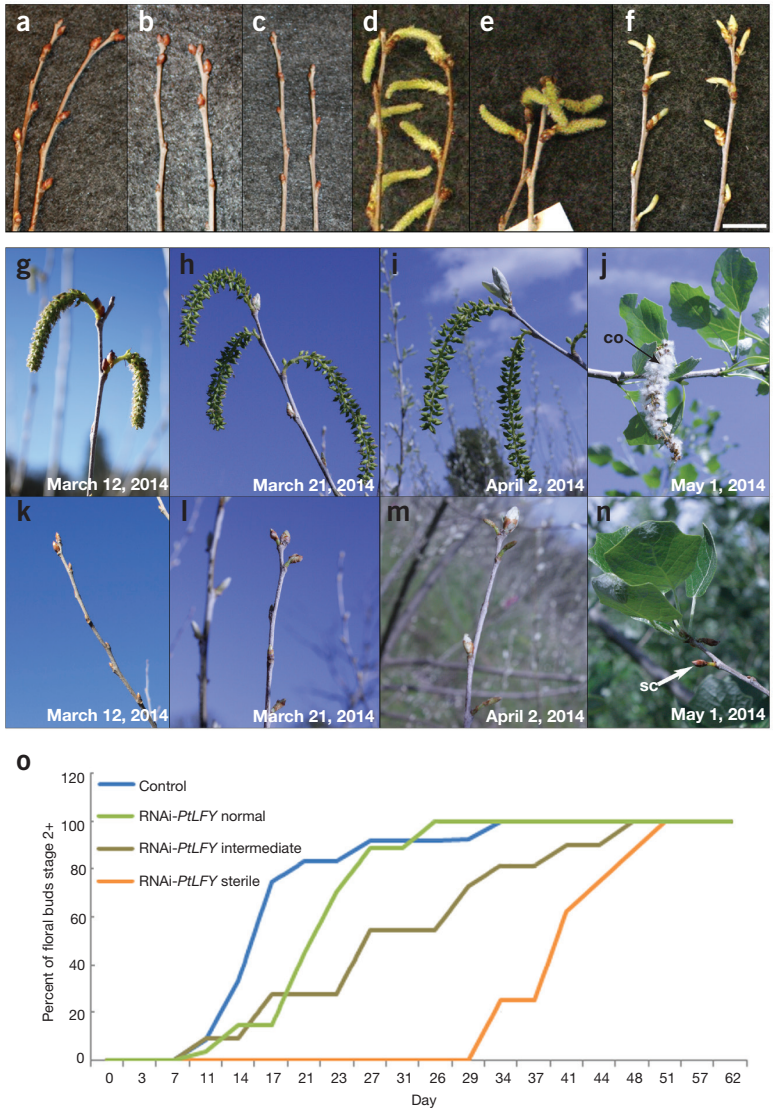

Figure 1 Small, late-opening catkins in sterile poplar events. Dormant buds were collected and screened to identify events with altered female catkin phenotypes. (**a**–**c**) Buds from control trees (**a**), buds from RNAi–*PtLFY* event 194 (**b**), buds from event 139-1 (**c**). (**d**,**e**) Catkins from control (**d**) and 13 of the RNAi–*PtLFY* events (**e**) fully flushed in 3 days (event 194 shown). (**f**) Catkins from events 17 and 139-1 fully flushed in 7 days (event 139-1 shown). Samples were imaged at the start of indoor incubation (**a**–**c**) and after full catkin emergence (**d**–**f**). Scale bar, 5 cm. (**g**–**n**) Catkins were imaged in field conditions from control trees (**g**–**j**) and RNAi–*PtLFY* trees with sterile catkins (**k**–**n**). Catkins were imaged over time in 2014 until control flowers fully matured and shed cotton (co). Small RNAi–*PtLFY* catkins observed on the same date were still encased in bud scales (sc). Catkins were photographed on the dates indicated in the lower right corner. (**o**) Floral bud opening over time beginning January 28, 2015 (day 0).

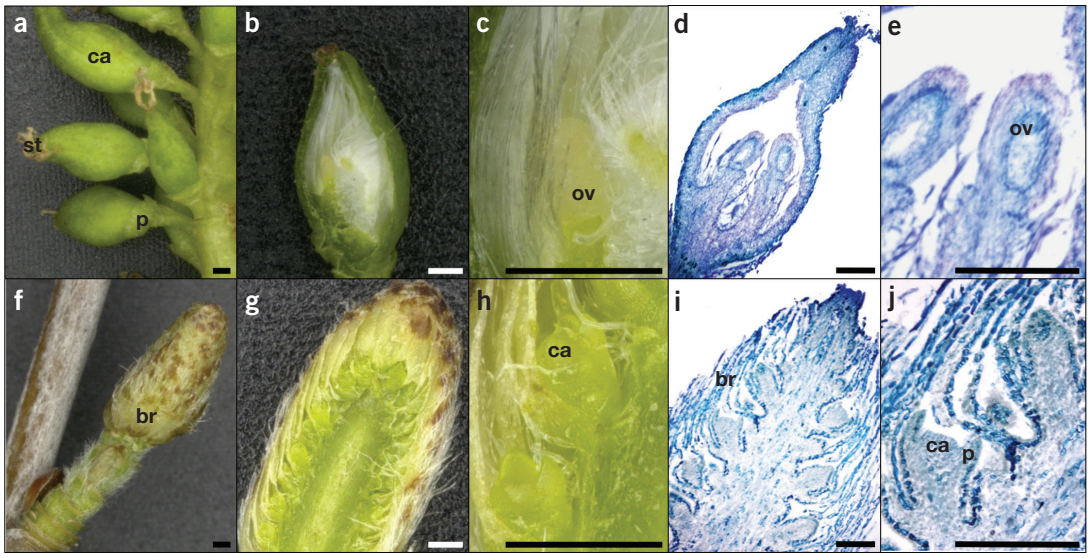

Figure 2 RNAi–*PtLFY* catkins were small and lacked stigmas or ovules. (**a**) Non-transgenic control catkins with carpels (ca) nested inside perianth cups (p) and topped with stigmas (st). (**b**,**c**) Hand-sectioned control carpels with cotton fibers and ovules (ov). (**d**,**e**) Wax-embedded sections of control carpels with ovules (ov). (**f**) RNAi–*PtLFY* catkins covered by bracts (br). (**g**,**h**) Hand-sectioned RNAi–*PtLFY* catkins with carpels (ca). (**i**,**j**) Wax-embedded sections of RNAi–*PtLFY* catkins with carpels and perianth cups (p). Catkins were collected for light microscopy April 9, 2014; control and RNAi–*PtLFY* catkins were collected for sectioning March 14, 2014. Scale bars, 500 μm.

for buds flushed indoors in 2016 from all 15 transgenic events, providing evidence for 3 years of trait stability in 17 and 139-1 (**Supplementary Fig. 4**).

The exterior and interior morphologies of field-flushed catkins were examined in both 2014 and 2015. Catkins from control trees contained well-formed carpels topped with stigmas. These carpels contained a large quantity of cotton and had readily visible ovules (**Fig. 2**). By contrast, catkins of RNAi–*PtLFY* events 17 and 139-1 collected on the same day as the control catkins were still covered by floral bracts, and had no externally visible carpels. Some of these catkins also had one or more subtending leaves, a phenotype not observed for RNAi–*PtLFY* events with normal flowers or for non-transgenic control trees (**Supplementary Fig. 5**). Dissection of catkins from events 17 and 139-1 revealed the presence of undeveloped carpels that lacked stigmatic structures. Sectioning of these carpels showed they were solid masses of cells, and lacked ovules (**Fig. 2**). Similar results were obtained in both 2014 and 2015. The small RNAi–*PtLFY* catkins collected in April 2015 from events 17 and 139-1 resembled wild-type catkins, but at a much earlier developmental stage.

Analysis of *P. alba LFY* (*PaLFY*) expression by quantitative real-time (qRT)-PCR showed that the two events with sterile catkins (17 and 139-1) had reduced *PaLFY* expression in developing floral buds collected in October 2014, compared with control floral buds (**Fig. 3**, $P < 0.05$). Quantification of *PaLFY* expression in expanded catkins collected in March showed that sterile catkins from the RNAi–*PtLFY* trees had significantly greater expression of *PaLFY* than was measured in control trees ($P < 0.05$). Quantification of *PaLFY* in an event with normal flowers (194) showed similar expression levels to control catkins. This expression difference between sterile and normal catkins may reflect the immature status of the sterile RNAi–*PtLFY* catkins. By March, control trees had catkins with fully developed carpels, while events 17 and 139-1 had catkins with undeveloped carpels that resembled an immature developmental stage in wild-type trees (**Fig. 2**)[17]. Typically, *PtLFY* is highly expressed in these early floral meristems, and its expression markedly decreases as floral organs initiate and differentiate[15]. The reduction in *PaLFY* expression may have delayed or arrested floral development, because *PaLFY* expression was insufficient for floral maturation.

We also assessed RNAi tree growth with and without spatial analysis to adjust for environmental variation within the plantation. Compared to analyses without spatial adjustment, spatial adjustment improved (lowered) the Akaike information criterion (AIC), a test used to judge model

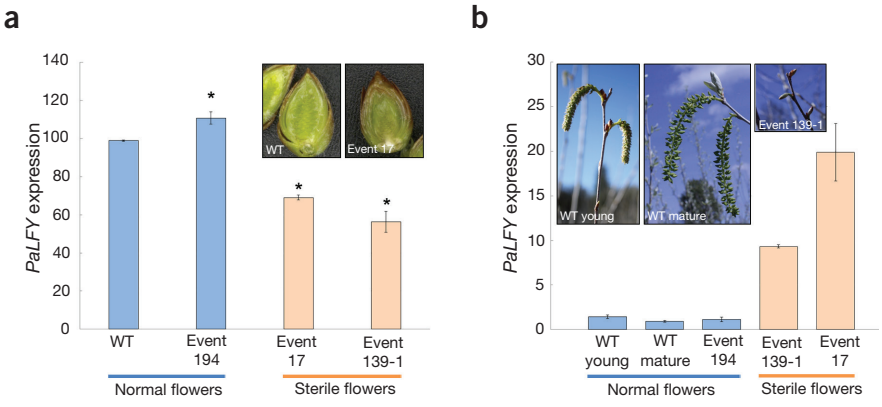

Figure 3 *PaLFY* expression in catkins. (**a**) Relative *PaLFY* transcript levels in developing floral buds collected October 3, 2014. *$P < 0.05$. (**b**) Relative *PaLFY* levels in young catkins collected March 21, 2014, and mature catkins collected April 2, 2014. Sterile RNAi–*PtLFY* catkins were collected March 21, 2014. Representative images of sampled tissues are shown. Error bars, mean ± s.e.m.

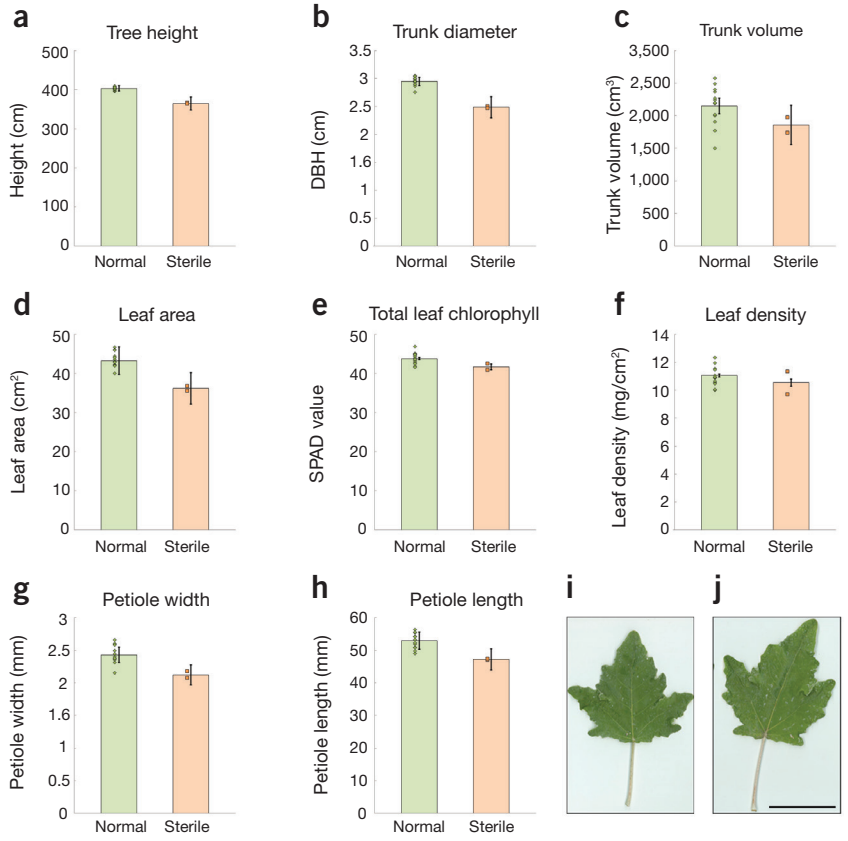

Figure 4 Growth phenotypes of RNAi–*PtLFY* trees. (**a**–**c**) Tree height (**a**), trunk diameter at breast height (DBH) (**b**), and volume index (**c**) of trees from 2015. (**d**–**h**) Leaf area (**d**), total leaf chlorophyll (**e**), leaf density (**f**), petiole width (**g**), petiole length (**h**) of leaves collected in 2015. Bars show the least-square means for flower treatments (normal flowers: green bars, 13 events; sterile flowers, orange bars, 2 events). Points (green diamonds, orange squares) represent the best linear unbiased predictions (BLUP values) for events within a treatment group. Error bars represent the standard errors of the treatment means. All values were derived using mixed model analyses in SAS. (**i**,**j**) Representative leaf scans of an RNAi–*PtLFY* leaf from an event with normal catkins (**i**) and an RNAi–*PtLFY* leaf from an event with sterile catkins (**j**). Scale bar, 5 cm.

fit, and lowered the standard errors of the flower treatment means. AIC values for tree height, trunk diameter at breast height (DBH), and volume index were 706.7, 192.4, and 1,030.2 before adjustment, and 685.1, 170.9, and 1,001.7 after spatial adjustment. After spatial adjustment, the s.e.m. for height, DBH, and volume index decreased by 16.0%, 13.3%, and 9.9%, respectively. Although the trees with normal catkins were slightly larger (16% greater volume index) than the trees with sterile catkins (**Supplementary Fig. 6**), none of these differences were statistically significant; the *P*-values for treatment differences were 0.297, 0.271, and 0.661 for height, DBH, and volume index. These results were robust to our analytical approach; all treatment differences were non-significant ($P > 0.05$) using analyses without spatial adjustment, and when a logarithmic transformation was applied (data not shown). Analysis of trunk volume by event showed no difference between events with normal and sterile catkins (**Supplementary Fig. 6** and **Supplementary Table 4**). The non-transgenic control trees, although measured, were not used in the statistical analyses described above because they did not undergo the transformation and organogenesis process *in vitro*. However, analogous analyses of growth traits that included the non-transgenic control trees showed no statistically significant differences in tree size among the sterile, normal, and non-transgenic control treatments (**Supplementary Fig. 6** and **Supplementary Table 5**).

The vegetative morphology and crown structure of sterile and normally flowering transgenic trees looked identical after visual inspection in the field. We also quantified leaf traits, including SPAD value (a measure of total leaf chlorophyll), leaf area, and leaf density (leaf mass per leaf area), and found no statistically significant differences between RNAi–*PtLFY* events with normal catkins and those with sterile catkins (**Fig. 4** and **Supplementary Table 6**). Therefore the reduction in reproductive development as a result of *LFY* suppression did not seem to be accompanied by a change in vegetative morphology, growth rate, or adaptability. However, we regard this observation as preliminary; our analysis included only two sterile events and a small number of replicates within events. Breeding-scale field trials, including analysis of more trees and events over additional years, and a more diverse array of poplar genotypes and test locations, are needed to confirm these findings.

In summary, we have shown that using RNAi to target the poplar homolog of *LFY* results in a decrease in inflorescence (catkin) size and loss of functional sexual organ development in field-grown trees while retaining normal vegetative development. *LFY* is highly conserved and functions very early in flower development, before the differentiation of floral organs[14]. Therefore, loss of *LFY* might have similar effects in male, female, and bisexual flowers of other angiosperm tree genera, and could be implemented using RNAi or genome editing. Because RNAi in field-grown poplar trees is generally stable[12], trees with sterile catkins should continue to show impaired reproductive growth over time. Even if some phenotypic reversion or vegetative spread were to occur, partial sterility would still result in much reduced potential for transgene flow (e.g., see ref. 18). Because of the strict regulatory requirements for field testing and commercial use of GE trees in much of the world[19]—which often hinges on the ecological and economic impacts from gene dispersal—the mitigation of sexual gene flow provided by *LFY* suppression could enable more rapid regulatory approval.

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METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. *PaLFY*: BankIt1922692, accession KX279885, *PtLFY*: Potri.015G106900, *ACT2*: Potri.001G309500.

AUTHOR CONTRIBUTIONS

S.H.S. oversaw the work and led the grant proposal that funded the research program; D.Z., A.L.K., K.A., J.H., H.L., R.M., O.S., C.M., M.D., S.R., A.M., and A.M.B. conducted the experiments; A.L.K., J.H., G.H.,

and A.M. analyzed the data; A.L.K. and S.H.S. wrote the manuscript.

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